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THE DEVELOPMENT OF ANTIBODIES IN THE SERUM OF PATIENTS RECOVERING FROM ACUTE LOBAR PNEUMONIA.

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Of the acute infectious diseases there is none which offers more interesting problems for investigation than acute lobar pneumonia. The remarkable phenomena occurring at the crisis have attracted particularly the attention of investigators. The very abrupt and radical change in the condition of the patient, "from perhaps a state of extreme hazard and distress to one of safety and comfort," indicates that some profound change must suddenly have taken place in the balance between the invading organisms and the defensive forces of the host. The numerous attempts to determine the nature of this change, to work out the mechanism by which recovery naturally occurs, have met with unexpected difficulties.

It is certain that the crisis is not due to an anatomical change in the diseased lung tissue; nor is it due to a sudden loss of virulence on the part of the organism. Rosenow[1] found no appreciable difference in the virulence of strains isolated by blood culture early or late in the disease. Luetscher[2] studied the comparative virulence of strains isolated from the sputum by plating, before, at, and after the crisis, and found no diminution in virulence for mice as long as actual sputum, from the lung, could be obtained.

Blood-culture studies have shown that death in pneumonia is usually due to the development of a general septicæmia, except in those cases in which some serious accidental complication develops. In favorable cases, on the contrary, the percentage of positive findings and the number of organisms present in the blood diminish as the crisis (or lysis) is approached. Rosenow[3] has shown that the number of viable cocci in consolidated lung tissue, obtained by repeated aspiration, becomes greatly reduced, or disappears during and after the crisis, but he did not demonstrate the mechanism by which they were destroyed.

It is generally agreed that the crisis is brought about by an increase in some of the defensive forces of the body, which prevents the unrestricted multiplication of the organisms; that it marks the development of an immunity which is due to, and associated with, the appearance of antibodies in the serum. That immune substances of some sort are found in human serum at the crisis was first demonstrated by G. & F. Klemperer.[4] They showed that the serum of convalescent patients, like that of immunized rabbits, will protect rabbits from otherwise fatal doses of virulent pneumococci. This has been confirmed by Roemer,[5] and by Neufeld and Haendel,[6,7] who found that some degree of protective power for mice was conferred by all of five sera investigated.

This protective power has since been denied by several other observers. Seligmann and Klopstock,[8] Boettcher[9] (in ten cases), and Strouse[10] (in eight cases), were unable to demonstrate any protective power for mice in postcritical sera. The

failure of the first writers mentioned was probably due to faulty technic, since the doses of culture used were too large, or were not accurately measured. Strouse used carefully graduated doses, but in most of his series, at least, used a virulent stock culture, which may have differed in its serum reactions from the strains infecting the patients whose sera were studied.

Eyre and Washbourn [11] were the first to demonstrate clearly such strain differences in pneumococci. They found that, whereas Pane's anti-pneumococcus serum in doses of 1 cc. protected rabbits from 1000 to 10,000 times the M. L. D. of four virulent strains, it did not protect from 10 M. L. D.'s of a fifth strain, otherwise indistinguishable from the other four. Bensaçon and Griffon,[12] Kindborg,[13] and Roemer [3] have also shown that a given serum differs markedly in its activity toward various strains of pneumococci, both in its agglutinating and protective power.

Neufeld and Haendel [14] have since shown that pneumococci can be subdivided into several groups, according to their reactions to immune sera, and their work has been confirmed by Cole and Dochez.[15] While all the strains in a given group, A, are about equally influenced by the serum of an animal immunized to one member of that group, a strain belonging to another group, B, will not be affected by this (group A) serum. That the failure of some to demonstrate protective power in posteritical serum may be due to such differences in the strain of organism used, is supported by the work of Dochez,[16] who has recently reported studies on 14 patients. Of ten cases in which an homologous organism was used, *i. e.*, one cultivated from the patient whose serum was being studied, nine possessed sera which showed a definite, though often slight, protective power. Of four sera tested with a stock culture, three showed no protective power whatever, and the fourth gave only a very feeble protection.

Previous attempts to demonstrate the nature of the immune bodies which confer this protection have led to contradictory results. The immunity to the pneumococcus which develops naturally in man after pneumonia is at best but slight, and of short duration. Because of the much higher degree of immunity that can be produced experimentally in animals, study of their sera has led to more definite results, and has suggested what may be looked for in human serum. Without going into detail, a review of the extensive literature on the subject [17] shows that the only antibodies which have been constantly demonstrated in significant quantity are substances which promote phagocytosis.

The importance of phagocytosis in immunity to the pneumococcus was first definitely shown by Issaeff.[18] He injected virulent pneumococcus cultures subcutaneously in normal and immunized rabbits. In the former he found that the organisms multiplied without restriction, leading to a septicæmia and to death of the animal. In the immunized rabbits, on the contrary, the organisms were ingested by leucocytes within six to ten hours; after 24 hours all the organisms had disappeared, and the animals recovered.

Mennes [19] first studied the action of immune serum and leucocytes on pneumococci in the test tube, using the method employed by Denys and Leclef [20] for streptococci. He found that, as in the animal body, virulent pneumococci were not phagocyted at all in normal serum, but were rapidly ingested and destroyed in immune serum. He found that the leucocytes of the immune animal, if washed free from the serum, were no more active than those of a normal animal. He did not find any direct bactericidal or antitoxic action on the part of the serum, and hence attributed the immunity to this acquired power of the serum to cause phagocytosis of virulent organisms.

His conclusions have been confirmed by Huber,[21] and by the extensive researches of Neufeld [22] and his co-workers. Neufeld further showed that the serum acts, not by stimulating the leucocytes, but by altering the microorganisms so as to make them susceptible to phagocytosis by normal leucocytes. Hence he gave to these antibodies the name bacteriotropines. They differ from the opsonines of normal serum in being thermostabile (60° C.). Since the bacteriotropine content of an immune serum, measured by the dilution method, runs closely parallel with its power of protecting animals, Neufeld attributes the immunity largely, if not entirely, to these substances. There can be little doubt that they play the leading rôle in the immunity of the horse and rabbit to the pneumococcus, but conclusions based on the properties of the serum of immune animals cannot be applied without further study to man.

The numerous early efforts to demonstrate specific bactericidal substances in significant quantities in human serum were unsuccessful. More recently Much [23] and Dold [24] have shown that normal human serum, and, to a much greater degree, citrated plasma (free from leucocytes), have a definite inhibitory action, and often a slight bactericidal action on virulent pneumococci. This activity is not present in the plasma of susceptible animals like the mouse, the rabbit, and the horse. To this Neufeld and Dold ascribe that degree of natural immunity which is possessed by man to virulent pneumococci, and they attribute to it the slight protective power for mice exerted by normal human serum.

I have repeated Dold's experiments with several specimens of normal human and rabbit serum and plasma, and with the serum and plasma of four patients convalescent from pneumonia (Cases VII, VIII, XII and XIII). The results were quite in accord with those of Dold. A reduction in the number of colonies in human plasma could usually be demonstrated, but this activity was not perceptibly increased in pneumonic plasma; nor was it more marked for the homologous strain of pneumococcus than for an heterologous strain. This direct bactericidal activity of the serum can certainly be a factor of only secondary importance in the immunity developed at the crisis.

Most writers have attributed the immunity developing in man to increased phagocytic activity. Attempts to demonstrate such activity have been limited largely to determinations of the opsonic index by the Wright method (*e. g.*, Rosenow,[25] Wolfe,[26] Boni,[27] Tunnicliff,[28] etc.). These observers

agree for the most part in finding a low opsonic index early in the disease, with a slight rise at the crisis; whereas, in unfavorable cases, there is a fall in the opsonic index before death. Even if we admit that the method is accurate enough to yield reliable results, the significance of such a rise in index is questionable. In such index determinations one must use a non-virulent or feebly virulent strain which is phagocytable in normal human serum. The organisms isolated from patients with pneumonia are almost invariably virulent and resistant to phagocytosis. This is true of strains cultivated from the sputum (Luetscher [24]), as well as from the blood (Rosenow,[23] etc.). A slight increase in the power of phagocyting avirulent organisms would, therefore, be of no evident benefit to the patient, unless, with this, there appeared the power of phagocyting virulent strains, such as that with which the patient is infected. As reported by the majority of observers, the serum of patients at the crisis is no more active than is normal serum in causing phagocytosis of such virulent strains (Rosenow,[23] Strouse,[10] Luetscher *).

Because of the similarity in the action of human postcritical serum and immune animal serum in protecting mice, Neufeld [7] thinks that the same antibodies are active in each. The crisis in man he explains as due to the appearance of antibodies (chiefly tropines) in the blood. The abruptness of the crisis he attributes to the fact that tropines are entirely inactive until they reach a certain *concentration* in the serum. They then suddenly become active, causing a rapid ingestion and destruction of all the hitherto non-phagocytable organisms. However, Neufeld does not offer any direct evidence proving that the active substances present in postcritical sera are tropines.

Foa [29] alone has reported that the serum of patients after crisis may cause phagocytosis of virulent organisms, not phagocytable in normal human serum. He found that serum heated at 56° C. was as active as fresh serum. He does not give his technic in detail further than that he followed Neufeld's method of using guinea-pig exudate leucocytes, and that he incubated the mixtures 45 minutes. The cultures he describes as "virulent," but the source of them is not stated. He gives no details as to the patients studied, nor does he expressly state that more than one individual case was tested.

Boettcher * has reported finding tropic activity in (heated) convalescent sera. Though she states that the cultures used were virulent strains obtained by blood cultures from patients with pneumonia, they could not have been highly virulent at the time the experiments were carried out, since they were phagocytable in normal serum. This increased activity of postcritical serum was therefore simply an increased power of ingesting a (relatively) avirulent phagocytable strain, and the significance of it is open to the same doubt as the reported increase in opsonic index, already described.

Boni [27] also reported finding increased phagocytic activity in heated serum at the crisis, as compared with normal heated serum. He describes the increase in heated serum (tropines) as more specific (as compared with staphylococci), but less marked than that in active serum (opsonines, as well as tropines). He also used phagocytable organisms, and his results are open to the same criticism as are those of Boettcher, for this reason.

Strouse [10] alone reports the use of homologous strains as well as stock cultures. He obtained entirely negative results with virulent non-phagocytable strains, and this was perhaps due to the fact that he used the sera in too high dilution (1 in 10 to 1 in 1000).

Rosenow [23] found that the bactericidal power of pneumonic defibrinated blood (for phagocytable strains) was greater than that of normal blood. This he found was due to phagocytosis. The increased activity bore no relation to the crisis, and was no more marked for the homologous than for an heterologous strain, but it was roughly proportional to the leucocytosis, and was attributed by him to a non-specific increase in the activity of the pneumonic leucocytes.

Tunnicliff [28] found at the crisis, by the plate method, an increase in the pneumococcidal power of the blood (serum and leucocytes) for avirulent strains only, which was roughly parallel with the rise in opsonic index. Eggers [30] found a similar increase for stock cultures of "moderate virulence." This he attributed to phagocytosis, but did not exclude the possibility of its being due in part to agglutination.

Because of these contradictory results it seemed desirable carefully to study the sera of a selected series of cases. The special object of the work was to determine the constancy with which antibodies appear at the crisis, and if possible to demonstrate their nature. It seemed probable that the failure of previous investigations was due to some common fundamental error in the methods employed. The work of Neufeld and Haendel suggested that this error was probably to be found in a difference between the strain of the organism used in the tests and that with which the patient was infected. Therefore, in all except one of the following series of cases the homologous virulent strain was employed. The activity of the serum of the patient after crisis (or lysis) was compared with that of normal serum, and in the last two cases with that of the same patient before the crisis, both as to its power passively to protect mice, and its power to promote phagocytosis in the test tube.

TECHNIC.

The cultures were isolated from the sputum of the patient as early as possible in the disease, in every instance before the temperature began to fall. The method used was that employed by Luetscher.[24] A single specimen of sputum, raised after a paroxysm of coughing, was expectorated into a sterile Petri dish, and immediately washed thoroughly three to eight times in sterile broth. A small particle of the washed sputum, or a few loops of broth from the last washing, were smeared with a sterile, bent glass rod over the surface of several human blood agar plates, and the latter incubated 24 hours. If the sputum was tenacious enough to permit thorough washing, practically pure cultures were easily obtained, containing at most four or five contaminating colonies on each plate. If

* Personal communication.

not tenacious enough to permit mouth contaminations to be washed entirely off, pure cultures could not be obtained. But if the great majority of the colonies (at least 80 per cent) were typical pneumococcus colonies, the culture was considered satisfactory, subcultures from several colonies were made, and their cultural reactions tested. Only typical strains were used. If culturally a typical pneumococcus was obtained, its phagocytability in normal human serum was determined, and, as a rule, its virulence for mice roughly tested. If non-phagocytable, and of such virulence that the M. L. D. for mice did not exceed 0.0002 cc. of a 24-hour serum-meat-infusion-broth culture, it was used without animal passage. This method of isolation was chosen in preference to the much easier one of animal inoculation in order to avoid a possible increase in virulence of the strain as a result of the animal passage. It was felt that the virulence of the strain isolated by plating would represent more accurately the virulence of the organism as it existed in the body of the patient than if animal inoculation were resorted to. A possible increase in virulence might conceivably hide a moderate degree of protective power in the serum. However, no comparative studies of the virulence of sputum strains isolated by the two methods were made.

From their isolation until used the strains were cultivated on rich, moist, human blood agar. Transfers were made at intervals of 1 to 3 or 4 days, care being taken to avoid desiccation. The results of these experiments indicated that, in the course of a few days to a few weeks, no change in the phagocytability of the organism occurred, nor was there any appreciable loss of virulence. That any loss of virulence can occur in the single generation on blood agar required for isolation, is scarcely conceivable.

In three cases growth was also obtained in blood cultures In two cases comparative tests of the virulence of blood and sputum strains, isolated at the same time, were made. In one case the blood strain, tested, however, 15 days after isolation, was distinctly less virulent than the sputum strain, tested 35 days after isolation, and again 52 days after isolation. In the third case both strains tested immediately were of equal and of very high virulence. The reliability of such sputum isolations is supported by the fact that in both cases the strains from the blood and sputum were identical in their serum reactions.

The blood was obtained by aspiration from the median basilic vein. The sterile serum was pipetted off after contraction of the clot, and stored on ice in the dark, without inactivation or the addition of a preservative. In the earlier cases but one specimen, taken one or two days after the crisis was examined. In later cases, where possible, several specimens, taken at intervals before and after crisis were tested.

In the first eleven cases the technic followed in the serum protection experiments was that described by Neufeld.[7] Each of a series of mice were injected into the peritoneal cavity with 0.2 cc. of patient's serum, a second series of control animals receiving the same quantity of normal serum. Three hours later all received intraperitoneal injections of a 24-hour serum-meat-infusion-broth culture of the homologous strain of pneumococci. The doses varied from 0.02 cc. to 0.000002 cc. in a volume of 0.2 cc. The mice were kept under observation for ten days or two weeks. As a rule, however, more definite, clear-cut results are obtained if the period of observation is shortened arbitrarily to four days, as recommended by Neufeld.[17] If the cultures are of the usual high virulence the control animals (receiving normal serum) die, as a rule, within 48 hours, and almost invariably they show a general septicæmia. A considerable proportion of the protected animals will outlive the controls, but will die later, after four to fifteen days. Cultures from the blood and peritoneum of such animals are almost invariably sterile. In some cases large hæmorrhages in the serous cavities were found, as described by Sprunt and Luetscher,[22] due to toxic degenerative changes in the elastic tissue of the large arteries. The animals in these cases seem to have overcome the infection and killed off the invading organisms, only to succumb to the late effects of the liberated toxines. While death is not prevented, the prolongation of life, and the sterile cultures after death, may be accepted as definite indications of some degree of protective power on the part of the serum. At best, the test is but a comparative quantitative one. Neufeld and others have shown that normal serum as used here often protects mice slightly but definitely from highly virulent cultures. In all cases autopsies were performed and the presence or absence of organisms in the blood and the peritoneum determined by smears, cultures, or by both.

In the last four cases Dochez's method was followed.[18] The serum (0.2 cc.) and each culture dilution (also 0.2 cc. in volume) were mixed in the test tube and after standing for about an hour the whole was injected intraperitoneally. This is undoubtedly better than the Neufeld method, and failure to use it may explain some of the early negative results.

Phagocytosis Tests.

The phagocytosis tests were carried out in every case with the virulent strain isolated during the acute stage from the sputum (or blood) of the patient whose serum was tested. With but two exceptions, discussed in detail later, they were not phagocytable in fresh normal human serum, under the conditions of the experiment. In every case several heterologous strains were also used, for the purpose of comparison. In particular, each serum showing any action on its homologous organism was also tested with the strain from every previous patient whose serum had shown any phagocytic activity. Fairly thick emulsions of the organism were made from 24-hour blood agar slants by rubbing up a loop of the growth in two or three drops of salt solution. The sera were used both in fresh condition and after inactivation for 30 minutes at 56° to 60° C. In a few instances the sera were also diluted 1 in 3, 1 in 10, 1 in 30, and 1 in 100. But experience soon showed that the activity was so slight that it usually disappeared entirely in a dilution of even 1 in 3. The leucocyte suspension was obtained either by defibrinating a few cubic centimeters of normal human blood or by mixing it in 1.5 per

cent sodium citrate solution, and washing three times in 0.85 per cent salt solution. The upper layers (the leucocytic "cream") were pipetted off, and thoroughly mixed, to insure a uniform suspension of leucocytes.

For the test equal volumes of serum, bacterial suspension, and leucocyte suspension were mixed and incubated at 37° C. in capillary pipettes, as in Wright's method.

After incubation smears of the mixtures were made by pulling a drop over the surface of one slide with the edge of a second narrower slide. The smears were fixed for two or three minutes in absolute methyl alcohol and stained for several minutes in methylene blue.

In every case, in addition to the preparations containing fresh, and those containing heated patient's serum, control preparations were made, some containing fresh normal serum, some heated normal serum, and some salt solution alone. In examining the smears counts were not made. Only gross differences easily recognizable after a rapid general survey of the preparation were regarded as of any significance. The degree of phagocytosis was described as absent (0), definite (+), strong (++), and maximal (+++). Unless the control preparations, containing normal serum, showed either a complete absence of phagocytosis, or at most a minimal degree of it (perhaps 10 or 20 pair in 50 leucocytes), phagocytosis in the test preparations was not regarded as significant. Some regard must be paid, however, to the time of incubation in order to get clear cut results. As a rule, the best preparations were obtained after 30 minutes incubation. If these were negative similar mixtures were incubated for one and two hours, but rarely was anything gained by the longer incubation. On the contrary, in several cases where, after 30 minutes incubation, the normal serum controls showed no phagocytosis and the postcritical serum strong phagocytosis, after two hours incubation the difference was much less definite, owing to a certain amount of spontaneous phagocytosis taking place in the controls. In other cases no phagocytosis took place, no matter how prolonged the incubation. A further drawback to prolonged incubation is the fact that not infrequently quite a marked degree of intracellular digestion of the organisms takes place, making them stain very poorly. Not infrequently, too, the leucocytes themselves showed signs of degeneration as if injured by the prolonged contact with the organisms. The activity of the leucocytes was always demonstrated by control mixtures containing a strain known to be easily phagocytable in normal serum.

This method was chosen in preference to the Neufeld method, in which guinea-pig leucocytes are used, because of the possible advantage of using homologous leucocytes, as Ungermann[33] has shown that under certain conditions sera may exert a bacteriotropic action only in the presence of homologous leucocytes.

In every instance the experiments were repeated once or twice on subsequent days with different specimens of normal serum and leucocytes to insure that the results were not accidental.

While this method gave satisfactory results as a routine procedure, it proved not to be delicate enough to demonstrate tropic activity in all cases. As above described, the mixture contains one volume of serum diluted with two parts of salt solution (bacterial and leucocyte suspension). As the sera which were active under such conditions lost their activity when diluted 1 in 3 (so that the actual serum concentration in the mixture was 1 in 9), it was felt that the method would prove more accurate if serum were employed instead of salt solution as the suspending medium.

In the one case in which there was an opportunity to test this modification, a positive result was obtained, whereas the regular method above described gave a negative result. Instead of suspending the culture in salt solution, it was suspended in a drop of postcritical serum, a control suspension being made in normal serum. Instead of using washed leucocytes, a suspension was prepared directly from the defibrinated blood of the patient and of a normal individual. This would permit sensitization of the cocci to take place in concentrated serum before being exposed to the action of the leucocytes, and would take advantage of any extra activity the latter might show when suspended in their own serum, as compared with a salt solution suspension. Equal parts of leucocyte suspension in serum and culture suspension in postcritical serum gave positive results, whereas normal leucocyte suspension and culture suspension in normal serum showed no trace of phagocytosis.

A further possible cause of failure might be the presence of inhibiting substances in the concentrated serum, as suggested by Neufeld. In negative cases, therefore, the organisms should also be digested in the serum and then washed before leucocytes are added.

Failure to use these modifications may be the cause of some of the earlier negative results.

Report of Cases.

Case I.—L. F., colored female, aged 13, was admitted to the hospital May 19, 1911, on the second day of the disease, with signs of consolidation of the right upper lobe, later extending to the middle and lower lobes as well. Blood culture on the fifth day gave a growth of pneumococcus. The strain used was plated from the sputum on the eighth day. The temperature fell by very protracted lysis from 102° on the sixth day to 100° F. on the sixteenth day. There was a slight elevation of temperature, from 99° to 100° F., for the following four weeks, with signs of delayed resolution. The patient was discharged August 2, with normal temperature and physical signs. The serum used was obtained May 28 (the eleventh day) and June 1 (the fifteenth day), both during lysis.

No.	Culture Dose.	Result.			
		May 28th.		June 1st.	
		Series A. Normal Serum.	Series B. Ser'm, 11th Day.	Series C. Normal Serum.	Series D. Ser'm, 15th Day.
1	5,000,000 pair.	+ 27 hours.	+ 6 days.	+ 4 days. Cult. 0.	Lived.
2	20,000,000 pair.	+ 24 hours.	+ 3 days.	+ 24 hours. Cult. +.	+ 5 days. Cult. 0.
3	80,000,000 pair.	+ 30 hours.	+ 6 days.	+ 5 days. Cult. 0.	Lived.
4	320,000,000 pair.	.....	.....	.....	+ days. Cult. +

In this one series a counted suspension of organisms from a blood agar slant was used. There was definite protective power shown by the serum obtained on the eleventh day, and also by that obtained on the fifteenth day.

The phagocytic activity of the serum was not tested.

CASE II.—R. G., colored male, 23 years old, entered the hospital November 27, 1911, on the third day of the disease, with signs of consolidation of the right middle lobe. The pneumococcus was plated from the sputum November 28. The temperature fell by lysis on the eighth to the eleventh days. The case was clinically a mild one. The serum was obtained December 4, on the tenth day, two days after the temperature began to fall, but two days before it reached normal.

RESULT OF TEST DECEMBER 4, 1911.

No.	Dose of Culture.	Series A. No Serum.	Series B. Serum of G—, December 4.
1	0.1 cc.	+ 24 hours.	.....
2	0.01 cc.	+ 24 hours.	.....
3	0.001 cc.	Lived.	+ 72 hours.
4	0.0001 cc.	+ 48 hours.	+ 60 hours.
5	0.00001 cc.	Lived.	+ 48 hours.
6	0.000001 cc.	+ 48 hours.	+ 60 hours.

There was no evidence of protective power, but rather an increased susceptibility to the culture, on the part of the treated mice. This probably is due to the use of serum obtained during the febrile stage. Washbourn [34] found that serum (blister fluid) of patients, during the acute stage of the disease, in six out of seven cases, distinctly increased the susceptibility of rabbits to a virulent pneumococcus culture.

The phagocytic activity of the serum was not tested.

CASE III.—C. W., colored male, 23 years old, was admitted to the hospital February 24, 1912, on the third day of the disease, with signs of consolidation of the right upper, middle, and lower lobes. Blood culture gave no growth. The temperature fell by protracted crisis on the ninth and tenth days. The serum was obtained ten days after the crisis. A stock culture of very high virulence was used, instead of an homologous strain. This may explain the complete failure to demonstrate any protective power.

The phagocytic activity of the serum was not tested.

RESULT MARCH 13, 1912.

No.	Culture Dose.	Series A. No Serum.	Series B. Normal Serum.	Series C. W— Serum, March 11.
1	0.0000001 cc.	+ 2 days.	.....	.....
2	0.0000003 cc.	+ 3 days.	.....	.....
3	0.000001 cc.	+ 2 days.	+ 2 days.	+ 2 days.
4	0.000003 cc.	.....	.....	+ 2 days.
5	0.00001 cc.	.....	+ 2 days.	+ 3 days.
6	0.00003 cc.	.....	.....	+ 2 days.
7	0.0001 cc.	.....	+	+ 1 day.
8	0.0003 cc.	.....	.....	+ 2 days.
9	0.001 cc.	.....	+ 2 days.	+ 2 days.
10	0.01 cc.	.....	.....	+ 1 day.

CASE IV.—F. C., white male, 23 years old, was admitted April 8, 1912, on the second day of the disease, with signs of consolidation of the right lower lobe. Blood cultures were sterile. The culture used was plated from the sputum on the third day of the disease. The temperature fell by crisis on (April 13) the seventh day. The serum was obtained three days after the crisis.

There was no protective power whatever. The doses used were a little too large.

The phagocytic activity of the serum was not tested.

RESULT APRIL 22, 1912.

No.	Culture Dose.	Series A. Normal Serum.	Series B. C— Serum, April 16.
1	0.2 cc.	+ 2 days.	+ 3 days.
2	0.02 cc.	+ 4 days.	+ 2 days.
3	0.002 cc.	+ 2 days.	+ 2 days.
4	0.0002 cc.	+ 2 days.	+ 2 days.
5	0.00002 cc.	+ 2 days.	+ 2 days.

CASE V.—C. P., white male, 19 years old, was admitted May 5, 1912, on the second day of the disease, with signs of consolidation of the right upper and left lower lobes. Blood culture showed no growth. The culture used was plated from the sputum on the second day. The temperature fell to normal by protracted crisis on the tenth and eleventh days. Serum was obtained May 14, the day after crisis.

RESULT MAY 14, 1912.

No.	Culture Dose.	Series A. Control Serum.	Series B. P— Serum of May 14.
1	0.2 cc.	+ 24 hours.	+ 48 hours.
2	0.02 cc.	+ 24 hours.	+ 48 hours.
3	0.002 cc.	Lived.	Lived.
4	0.0002 cc.	+ 48 hours.	Lived.
5	0.00002 cc.	+ 48 hours.	Lived.

There was definite protection from ten and from a hundred times the dose fatal for control mice. The survival of one control mouse, which received a larger dose than two of the others which died, illustrates an irregularity not infrequently met with, and probably referable to an individual peculiarity of this mouse.

PHAGOCYTOSIS TEST MAY 16.

(1) Washed normal leucocytes + P— culture suspension + normal serum. Phagocytosis 0.

(2) Leucocytes + P— culture + P— serum (May 14). Phagocytosis ++ (strong).

The serum thus showed definite phagocytic activity as compared with normal serum.

CASE VI.—A. H., white male, aged 19 years, was admitted December 7, 1912, on the third day of the disease, with consolidation of the right middle and lower lobes. Blood culture gave no growth. The culture used was plated from the sputum on the fourth day. The temperature fell by crisis very abruptly on the seventh day (December 11). The serum was obtained December 13, two days after crisis.

RESULT DECEMBER 14, 1912.

No.	Culture Dose.	Series A. Control Serum.	Series B. H— Serum of December 13.
1	0.02 cc.	+ 2 days.	+ 18 hours.
2	0.002 cc.	+ 2 days.	+ 2 days.
3	0.0002 cc.	+ 2 days.	+ 2 days.
4	0.00002 cc.	+ 2 days.	+ 2 days.
5	0.000002 cc.	+ 2 days.	+ 2 days.

There was not the slightest evidence of protection.

Phagocytosis tests were also negative.

(1) Washed leucocytes + H— culture + normal serum = 0.

(2) Washed leucocytes + H— culture + H— serum = almost 0.

The difference in the preparations was so slight as to be negligible.

CASE VII.—S. T., white male, aged 18 years, was admitted March 8, 1913, on the third day, with signs of consolidation of the right lower lobe. Blood culture on March 10 showed no growth. The culture used was plated from the sputum on the fourth day. The temperature fell by crisis on the seventh day (March 12) to 99° F.,

but continued slightly elevated (99° to 100° F.) for ten days. The serum was obtained March 13, two days after crisis.

RESULT MARCH 13, 1913.

No.	Culture Dose.	Series A. Control Serum.	Series B. T—Serum.
1	0.2 cc.	+ 6 days (cult. positive).	+ 6 days.
2	0.02 cc.	Lived.	+ 4 days.
3	0.002 cc.	+ 3 days.	+ 6 days.
4	0.0002 cc.	+ 5 days.	Lived.
5	0.00002 cc.	+ 6 days.	+ 5 days.
6	0.000002 cc.	Lived.	+ 5 days.

No protective power was shown.

As the culture was of low virulence and killed slowly, the virulence was raised by passage through 8 mice, and the tests repeated, using the same specimen of serum.

RESULT JUNE 16, 1913.

No.	Culture Dose.	Series A. Normal Serum.	Series B. T—Ser., March 13.
1	0.02 cc.	+ 24 hours.	+ 24 hours.
2	0.002 cc.	+ 48 hours.	+ 24 hours.
3	0.0002 cc.	+ 48 hours.	+ 24 hours.
4	0.00002 cc.	Lived.	+ 24 hours.
5	0.000002 cc.	Lived.	+ 24 hours.

A control mouse receiving T—'s serum without culture lived. In this series the sera were mixed with culture dilutions and the mixtures were injected into the mice. The patient's serum (which might conceivably have weakened in three months) not only offered no protection, but favored infection, as compared with the normal serum controls. This failure to show protection may be due to the fact that the serum was taken before the temperature had quite reached normal.

Phagocytosis tests were also negative. T—'s culture was not phagocyted at all by washed leucocytes, either in normal serum, or in T—'s serum of March 13.

CASE VIII.—F. P., colored male, 19 years old, was admitted March 11, 1913, on the fourth day, with consolidation of the left upper lobe. The pneumococcus was cultivated from the blood on the fifth day, about 5 colonies per cubic centimeter. The strain used was plated from the sputum on the fifth day. The temperature fell by crisis on the tenth day (March 17). There was a slight persistent postcritical elevation of temperature, with persisting signs of consolidation in the left upper lobe, which were regarded clinically as indicating delayed resolution. They subsided gradually after ten days. The serum used was obtained March 18, the day after crisis.

RESULT MARCH 22, 1913.

No.	Culture Dose.	Series A. Normal Serum.	Series B. P— Serum, March 18.
1	0.02 cc.	+ 1 day culture +	+ 1 day culture +
2	0.002 cc.	+ 1 day culture +	+ 1 day culture +
3	0.0002 cc.	+ 2 days culture +	+ 2 days culture +
4	0.00002 cc.	+ 1 day culture +	+ 3 days culture sterile.
5	0.000002 cc.	+ 1 day culture +	+ 5 days culture sterile.

Some protection was shown. The mice Nos. 4 and 5, in Series B, outlived the controls, and overcame the infection, as shown by sterile cultures, whereas the controls all showed a septicæmia.

Phagocytosis tests were negative. The strain P— was not phagocyted by washed leucocytes, either in fresh normal serum, or in P—'s postcritical serum.

CASE. IX.—L. T., colored female, 20 years of age, was admitted March 17, on the fifth day, with signs of consolidation of the right middle and lower lobes. Blood culture on admission gave no growth. The culture was plated from the sputum on the fifth day. The temperature fell by protracted crisis on the sixth and seventh days. Serum was obtained on March 20, the day after crisis.

RESULT APRIL 11, 1913.

No.	Culture Dose.	Series A. Normal Serum.	Series B. T— Serum, March 20.
1	0.02 cc.	+ 24 hours culture +	+ 24 hours culture +
2	0.002 cc.	+ 24 hours culture +	+ 48 hours culture +
3	0.0002 cc.	+ 24 hours culture +	+ 48 hours culture +
4	0.00002 cc.	+ 48 hours culture +	+ 3 days culture sterile.
5	0.000002 cc.	+ 48 hours culture +	+ 3 days culture sterile.

There is a slight degree of protective power shown in that two of the protected mice outlived the controls, and gave sterile cultures.

Phagocytosis tests were negative. The strain T— was not phagocyted by washed leucocytes, either in normal serum, or in T—'s postcritical serum.

CASE X.—J. A., colored male, aged 29, was admitted March 24, 1913, on the third day of the disease, with consolidation of the right upper lobe. Blood culture on admission gave no growth. The culture was plated from the sputum on the fourth day. The temperature fell by crisis on the fifth day (March 26). Serum was obtained March 27, the day after crisis.

RESULT APRIL 11, 1913.

No.	Culture dose.	Series A. Normal Serum.	Series B. A— Serum, March 27.
1	0.02 cc.	+ 7 days.	+ 3 days culture 0.
2	0.002 cc.	+ 3 days culture +	+ 6 days.
3	0.0002 cc.	+ 6 days.	+ 4 days.
4	0.00002 cc.	+ 4 days culture +	+ 3 days culture 0.
5	0.000002 cc.	+ 4 days.	+ 3 days culture 0.

There was no evidence of protective power. Attempts to increase the virulence of the strain by passage through six mice were unsuccessful.

There was no demonstrable increase in phagocytic activity. The strain A— was not phagocyted at all by washed leucocytes, either in normal serum, or in A—'s postcritical serum.

CASE XI.—W. R., colored male, aged 27, was admitted March 7, 1913, on the fourth day of the disease, with consolidation of the right upper and middle lobes. The pneumococcus was cultivated from the blood on admission, one colony per cubic centimeter (strain R— blood). In addition a sputum strain was used, isolated by plating, on the fourth day. The temperature fell by lysis on the eighth to the tenth days. The serum was obtained March 14, the day after the temperature became normal.

RESULT MARCH 22, 1913, USING STRAIN R— BLOOD.

No.	Culture Dose.	Series A. Normal Serum.	Series B. R— Serum, 11th day.
1	0.02 cc.	+ 2 days culture 0.	+ 10 days.
2	0.002 cc.	+ 8 days culture 0.	+ 10 days.
3	0.0002 cc.	+ 6 days culture 0.	+ 2 days culture +
4	0.00002 cc.	+ 5 days culture 0.	Lived.
5	0.000002 cc.	+ 3 days	+ 9 days.

The protected animals on the average outlived the controls. But the virulence of the culture (after two weeks cultivation on blood agar) was too low to give definite results.

RESULT APRIL 10, 1913.

No.	Culture Dose.	Series A. Normal Serum.	Series B. R— Serum.	No Serum.
1	0.02 cc.	+ 1 day culture +	+ 4 days culture 0.	.....
2	0.002 cc.	+ 1 day culture +	+ 4 days culture 0.	+ 24 hours.
3	0.0002 cc.	+ 1 day culture +	Lived.	.....
4	0.00002 cc.	+ 6 days culture 0.	+ 2 days culture 0.*	+ 48 hours.
5	0.000002 cc.	Lived.	+ 6 days culture 0.	.....

* This mouse died of a large intrapleural hæmorrhage.

Second Test: Here the strain isolated from the sputum was used. It had been grown for five weeks on blood agar, without passage through animals.

This series showed well-marked protection in the survival of the treated mice for a considerably longer period than the controls, and in their yielding sterile cultures, in contrast with the septicæmias in the controls.

The phagocytosis tests will be described in connection with Case XII.

CASE XII.—C. S., colored male, aged 34, was admitted to the hospital March 18, 1913, on the sixth day, with signs of consolidation of the left lower lobe. Blood culture on March 20 was sterile. The culture used was plated from the sputum on the seventh day. The temperature fell by crisis on the tenth day, though there was a persistent elevation of temperature to about 100° F. for two weeks. This was associated with an encapsulated effusion (X-ray) in the left lower chest, which finally was absorbed spontaneously, and the patient was discharged well April 14. The serum was obtained March 22, the day of crisis.

RESULT APRIL 16, 1913.

No.	Culture Dose.	Series A. Normal Serum.	Series B. S— Serum, 10th day.
1	0.02 cc.	+ 2 days.	Lived.
2	0.002 cc.	+ 2 days.	+ 2 days.
3	0.0002 cc.	+ 2 days.	Lived.
4	0.00002 cc.	+ 1 day.	+ 6 days.
5	0.000002 cc.	Lived.	+ 7 days.

There was definite and fairly strong protective power demonstrated.

PHAGOCYTOSIS TESTS WITH SERA OF CASES XI AND XII.

No.	Leucocytes.	Cultures Used.	Serum Used.	Degree of Phagocyto's.
1	Washed leucocytes +	R— blood +	Normal serum.	0
2	Washed leucocytes +	R— blood +	R— serum (fresh).	+ + +
3	Washed leucocytes +	R— sputum +	Normal serum.	0
4	Washed leucocytes +	R— sputum +	R— serum (fresh).	+ + +
5	Washed leucocytes +	R— sputum +	R— serum (heated to 56°C.).	+ +
6	Washed leucocytes +	R— sputum +	R— serum (diluted 1 in 3).	Almost 0.
7	Washed leucocytes +	R— sputum +	R— serum (diluted 1 in 3 + normal serum).	Almost 0.
8	Washed leucocytes +	R— sputum +	R— serum (26 days old)	+ + +
9	Washed leucocytes +	R— sputum, after passage through a mouse.	R— serum.	+ + +
10	Washed leucocytes +	S— sputum +	R— serum.	0
11	Washed leucocytes +	P— sputum +	R— serum.	0
12	Washed leucocytes +	T— sputum +	R— serum.	0
13	Washed leucocytes +	A— sputum +	R— serum.	0
14	Washed leucocytes +	S— sputum +	Normal serum.	0
15	Washed leucocytes +	S— sputum +	S— serum (fresh).	+ + +
16	Washed leucocytes +	S— sputum +	S— serum (heated to 56°C.).	+
17	Washed leucocytes +	S— sputum +	S— serum (diluted 1 in 3).	0
18	Washed leucocytes +	R— sputum +	S— serum.	0
19	Washed leucocytes +	T— sputum +	S— serum.	0
20	Washed leucocytes +	A— sputum +	S— serum.	0
21	Washed leucocytes +	S— sputum +	S— serum (20 days old).	+ +
22	Washed leucocytes +	S— sputum +	S— serum (32 days old).	Almost 0.
23	Washed leucocytes +	R— sputum +	R— serum (40 days old).	+ +
24	Washed leucocytes +	R— sputum +	R— serum (50 days old).	+

The results of several series of tests are combined in the table above. They show that the postcritical serum of each of these two patients caused active phagocytosis of the homologous virulent strain, which, in each case, was not phagocyted in normal human serum. Neither serum caused any phagocytosis whatever of the strain isolated from the other patient, nor of several other heterologous strains. These two strains, therefore, differed sharply in their serum reactions, as tested in vitro, and presumably might fall into different groups, though they were practically identical culturally. To determine whether the sera showed the same specificity in strain in their power of protecting mice, as in their phagocytic activity in vitro, the following crossed protection tests were carried out, the same specimens of serum being used as in earlier tests. The cultures used were R— sputum, and S— sputum, after it had been passed through one mouse.

RESULT APRIL 26, 1913.

No.	Culture R—. Culture Dose.	Series A. Normal Serum.	Series B. R— Serum.	Series C. S— Serum.
1	0.02 cc.	+ 1 day culture +	+ 4 days culture 0.	+ 1 day cult. +
2	0.002 cc.	+ 1 day culture +	Lived.	+ 2 d'ys cult. +
3	0.0002 cc.	+ 2 days culture +	Lived.	+ 2 d'ys cult. +
4	0.00002 cc.	+ 2 days culture +	+ 5 days culture 0.	+ 2 d'ys cult. +
5	0.000002 cc.	Lived.	Lived.	+ 1 day cult. +

No.	Culture S—. Culture Dose.	Series D. Normal Serum.	Series E. R— Serum.	Series F. S— Serum.
1	0.02 cc.	+ 2 days culture +	+ 2 days cult. 0.	Lived.
2	0.002 cc.	+ 3 days culture +	+ 2 days cult. +	+ 2 days cult. 0.
3	0.0002 cc.	+ 3 days culture +	+ 2 days cult. +	+ 2 days cult. 0.
4	0.00002 cc.	+ 3 days culture +	+ 2 days cult. 0.	Lived
5	0.000002 cc.	+ 5 days culture +	+ 2 days cult. +	+ 2 days cult. 0.

These tests showed that the protective power for mice of these sera ran parallel with the phagocytic activity in the test tube. R— serum protected very well from the homologous strain, but did not at all from S—. S—'s serum, on the other hand, did not show the least protective power toward R—, but did protect definitely, though rather feebly, from the homologous strain, S—.

CASE XIII.—W. D., colored male, 17 years old, was admitted April 24, 1913, on the fifth day, with consolidation of the right lower lobe. Blood culture on admission was negative. The culture used was plated from the sputum April 24. The temperature fell by crisis on the sixth day. Serum was obtained May 3, ten days after crisis.

RESULT MAY 16, 1913.

No.	Culture Dose.	Series A. Normal Serum.	Series B. D— Serum.
1	0.02 cc.	+ 2 days culture +	Lived.
2	0.002 cc.	+ 3 days culture +	+ 4 days culture 0.
3	0.0002 cc.	+ 1 day culture 0.	Lived.
4	0.00002 cc.	+ 2 days culture 0.	Lived.
5	0.000002 cc.	+ 2 days culture 0.	+ 1 day culture 0.

There was well marked protective power manifested.

Phagocytosis tests May 3, 1913. As the ordinary method gave negative results, special methods, already described, were used.

No.	Leucocytes.	Culture Strain Suspended In.	Serum.	Result Degree of Phagocytosis.
1	Normal blood.	D— in normal serum.	Normal.	0
2	D— blood.	D— in D— serum.	D—.	+ + +
3	Normal blood.	D— in D— serum.	D—.	+ +
4	Normal blood.	D— in salt solution.	D—.	0
5	D— blood.	R— in salt solution.	D—.	0
6	D— blood.	S— in salt solution.	D—.	0
7	D— blood.	P— in salt solution.	D—.	0
8	D— blood.	N— in salt solution.	D—.	0

By suspending the cocci directly in serum and using defibrinated blood in place of washed leucocytes, marked phagocytosis of D—

strain was demonstrated in his own serum; but none whatever in normal serum. The patient's leucocytes seemed to be slightly more active than normal leucocytes: but the essential factor in this activity was in the serum. The serum had no effect on four heterologous strains treated, three of which had been phagocytable in the postcritical serum of the patient from whom they were cultivated.

CASE XIV.—R. C., white male, aged 49, was admitted May 20, 1913, on the first day of the disease, later developing signs of consolidation of the left upper and lower lobes. Blood culture on the second day was sterile. The culture was plated from the sputum on the second day. It was of the streptococcus mucosus type. The temperature fell abruptly (pseudocrisis) on the fifth day, but rose again, and fell by lysis, to normal on May 30. Serum was obtained May 21, May 23, May 26 (during lysis), and May 31 (after complete lysis).

RESULT MAY 27, 1913.

No.	Culture Dose	Series A. Norm'l Ser'm	Series B. C—, 5-21.	Series C. C—, 5-23.	Series D. C—, 5-26.	No Serum.
1	0.02 cc.	+ 24 hours.	+ 18 hours.	+ 18 hours.	+18 hours.	+24 hours.
2	0.002 cc.	+ 18 hours.	+ 24 hours.	+ 18 hours.	+24 hours.	.....
3	0.0002 cc.	+ 24 hours.	+ 42 hours.	+ 42 hours.	+42 hours.	+24 hours.
4	0.00002 cc.	+ 42 hours.	+ 42 hours.	+ 42 hours.	+42 hours.	.....
5	0.000002 cc.	+ 24 hours.	+ 24 hours.	+ 18 hours.	+24 hours.	+48 hours.

Cultures positive.

RESULT JUNE 16, 1913.

No.	Culture Dose.	Series E. Normal Serum.	Series F. C— Serum, 5-31.
1	0.02 cc.	+ 19 hours culture +	+ 18 hours culture +
2	0.002 cc.	+ 42 hours culture +	+ 42 hours culture +
3	0.0002 cc.	+ 42 hours culture +	Lived.
4	0.00002 cc.	+ 66 hours culture +	Lived.
5	0.000002 cc.	+ 42 hours culture +	+ 66 hours culture 0.

There was definite, though not strong, protective power, but only in the one specimen, taken after the temperature permanently had reached normal. Some of the negative results obtained in previous cases are probably due to failure to test the serum at repeated intervals, as has been pointed out by Dochez.

PHAGOCYTOSIS TESTS.

No.	Leucocytes.	Culture Strain Suspended In.	Serum.	Result.
1	Normal blood.	C— normal serum.	Normal.	Almost 0.
2	Normal blood.	C— C— serum, 5-21.	C—, 5-21.	Almost 0.
3	Normal blood.	C— C— serum, 5-23.	C—, 5-23.	Almost 0.
4	C— blood, 5-23.	C— C— serum, 5-23.	C—, 5-23.	Almost 0.
5	Normal blood.	C— in salt solution.	Normal.	0
6	Normal blood.	C— in salt solution.	C—, 5-26.	+ +
7	C— blood, 5-26.	C— in salt solution.	C—, 5-26.	+ + +
8	C— blood, 5-26.	P— in C— serum, 5-26.	C—, 5-26.	0
9	C— blood, 5-26.	D— in C— serum, 5-26.	C—, 5-26.	0
10	C— blood, 5-26.	S— in C— serum, 5-26.	C—, 5-26.	0
11	Washed leucocytes.	C— in salt solution.	Normal.	0
12	Washed leucocytes.	C— in salt solution.	C—, 5-26.	+ +
13	Washed leucocytes.	C— in salt solution.	C— (heated at 56°C.).	+ + +
14	Washed leucocytes.	C— in salt solution.	C—, 5-26 (diluted 1-3).	+ +
15	Washed leucocytes.	C— in salt solution.	(Diluted 1-10.)	0
16	Washed leucocytes.	C— in salt solution.	C—, 5-31.	+ to + +
17	Washed leucocytes.	P— bl. in salt solution.	C—, 5-26.	0
18	Washed leucocytes.	W— bl. in salt solution.	C—, 5-26.	0

This table shows that the culture C— was not phagocyted at all in normal serum, nor in the serum of the patient early in the disease. With the fall in temperature, the serum acquired the power of promoting phagocytosis of this one corresponding strain, but not of five other heterologous strains, all of which had been shown to be susceptible to the action of their own homologous serum. This power appeared at a time when efficient protective power had not yet appeared. It was more marked than usual, in being evident in a 1 to 9 dilution of serum, but not in a 1 to 30 dilution.

CASE XV.—R. P., colored male, 24 years old, was admitted May 28, 1913, on the third day of the disease, with consolidation of the right upper and middle, and left upper lobes. The pneumococcus was cultivated from the blood on the third day, and again on the fourth day, when 175 colonies per cubic centimeter appeared on the plates. A sputum strain was also obtained by plating on the third day. Death occurred on the sixth day. The serum was obtained on the third day. This man lived in the same house as did Case XVI, and was taken sick two days later than he.

CASE XVI.—D. W., colored male, 27 years old, was admitted May 28, on the fifth day, with consolidation of the right lower lobe. Blood culture on admission was negative. The culture used was plated from the sputum on the fifth day. The temperature fell by crisis on the eighth day. Serum was obtained on the fifth day (May 28), eighth day (crisis, May 31), and ten days after crisis (June 10).

PRELIMINARY VIRULENCE TESTS (MICE).

No.	Dose.	Series A. P— Blood.	Series B. P— Sputum	Series C. W—.
1	0.000002 cc.	+ 48 hours.	+ 48 hours.	.....
2	0.00002 cc.	+ 48 hours.	+ 48 hours.	+ 48 hours.
3	0.0002 cc.	+ 48 hours.	+ 48 hours.	.....
4	0.002 cc.	+ 24 hours.	+ 24 hours.	.....
5	0.02 cc.	+ 24 hours.	+ 24 hours.	.....

All three strains were of high virulence for mice; and the blood and sputum strains of P— were of equal virulence.

PHAGOCYTOSIS TESTS.

No.	Leucocytes.	Culture Strain Suspended In.	Serum.	Result. Deg. of Phagocytosis.
1	Washed, normal +	P— sputum in salt sol. +	Normal human.	+ +
2	Washed, normal +	P— sputum in salt sol. +	P—, 5-28.	+ +
3	Washed, normal +	P— blood in salt sol. +	Normal human.	+ +
4	Washed, normal +	W— in salt solution +	Normal human.	+ +
5	Washed, normal +	W— in salt solution +	W—, 5-28.	+ +
6	Washed, normal +	P— sputum in salt sol. +	Normal human (heated to 56°C.).	0
7	Washed, normal +	P— blood in salt sol. +	Normal human (heated to 56°C.).	0
8	Washed, normal +	W— in salt solution +	Normal human (heated to 56°C.).	0
9	Washed, normal +	P— sputum in salt sol. +	Norm. rabbit, fresh.	0
10	Washed, normal +	P— sputum in salt sol. +	Norm. mouse, fresh.	0
11	Washed, normal +	P— blood in salt sol. +	Norm. rabbit, fresh.	0
12	Washed, normal +	P— blood in salt sol. +	Norm. mouse, fresh.	0
13	Washed, normal +	W— in salt solution +	Norm. mouse, fresh.	0
14	Washed, normal +	W— in salt solution +	Norm. rabbit, fresh.	0
15	Washed, normal +	P— blood in salt sol. +	P— serum, 56°C.	0
16	Washed, normal +	P— blood in salt sol. +	W—, 5-31, 56°C.	+ +
17	Washed, normal +	W— in salt solution +	P— serum, 56°C.	0
18	Washed, normal +	W— in salt solution +	W— ser., 5-28, 56°C.	0
19	Washed, normal +	W— in salt solution +	W— ser., 5-31, 56°C.	+ + +
20	Washed, normal +	W— in salt solution +	W— ser., 6-10, 56°C.	+ to + +
21	Washed, normal +	W— in salt solution +	W— ser., 5-31, 56°C. (diluted 1 in 3).	0
22	Washed, normal +	C— in salt solution +	W— ser., 5-31, 56°C.	0
23	Washed, normal +	Pr— in salt solution +	W— ser., 5-31, 56°C.	0
24	Washed, normal +	Neufeld PnI in salt sol. +	W— ser., 5-31, 56°C.	0
25	Washed, normal +	R— in salt solution +	W— ser., 5-31, 56°C.	0
26	Washed, normal +	S— in salt solution +	W— ser., 5-31, 56°C.	0
27	Washed, normal +	D— in salt solution +	W— ser., 5-31, 56°C.	0

A study of the tests which were repeated several times with identical results, and which are summarized in the table above, reveals some very interesting facts. All three strains, which had shown a high virulence for mice, were readily phagocytable in fresh normal human serum. These were the only strains met with, isolated during the acute stage of the disease, which were phagocytable at all (when isolated) in fresh

normal serum, and, at first sight, it would seem probable that they were to be regarded as exceptional cases, where a low virulence for man did not run parallel with the high virulence for laboratory animals. This view is supported by the fact that none of the three strains were phagocytable in fresh normal rabbit, or mouse serum. As regards the sputum strains the objection might be raised (improbable though it be, since the cultures on the plates were practically pure) that the colonies subcultured were accidental contaminations from the mouth, and did not represent the infecting strain. Such an objection cannot be raised to the blood culture isolation, which behaved identically like the other two. It seems highly improbable, too, that a strain is to be regarded as avirulent for man, when it is isolated from the blood of a patient critically ill two or three days before death. This would seem to be the best available criterium of virulence for man. There was no possibility of a change taking place in the culture after isolation, since these results were obtained in tests on May 30, after but one generation on artificial media. While a single exception is not sufficient to overthrow a rule, it suggests the advisability of using greater caution in regarding the virulence of a strain for man as always running inversely parallel with its phagocytability in human serum.

While there was no conspicuous difference between the phagocytic activity of this patient's serum and that of normal serum when fresh, striking differences in their behavior appeared after inactivation. All three strains were quite resistant to phagocytosis in inactive normal serum (heated at 56° C., or after standing one week on ice), and were likewise not affected by the heated serum of either patient at the height of the disease. But the heated serum of the patient who recovered (W—), obtained at the crisis and (to a slightly less degree) also that obtained ten days after crisis, caused active phagocytosis of the homologous strain and also of the strains of P—, who probably had been infected directly by W—. It did not influence any of the other heterologous strains tested, in particular not that from C—, whose serum had caused phagocytosis of his own strain, but not of the strains from P— or W—.

In order to test the parallelism of the phagocytic activity and the protective power of the sera, the following series of mice were inoculated, using mixtures of serum and diluted culture.

No.	Dose of Culture P— Blood.	Series A. Normal Serum.	Series B. P— Serum, 5-28.	Series C. W— Serum, 5-31.
1	0.02 cc.	+ 18 hours cult. +	+ 18 hours cult. +	Lived.
2	0.002 cc.	+ 20 hours cult. +	+ 22 hours cult. +	Lived.
3	0.0002 cc.	+ 22 hours cult. +	+ 40 hours cult. +	Lived.
4	0.00002 cc.	+ 40 hours cult. +	+ 40 hours cult. +	+ 20 hours cult. +
5	0.000002 cc.	+ 40 hours cult. +	+ 40 hours cult. +	Lived.

There was efficient protection by W—'s serum obtained at crisis, for the strain P— blood, but none by P—'s serum, obtained during the acute stage of the disease, three days before death. The protective power here ran parallel with phagocytic activity.

No.	Dose of Culture C—.	Series A. Normal Serum.	Series B. W— Serum, 5-31.
1	0.002 cc.	.....	+ 24 hours.
2	0.0002 cc.	.....	+ 40 hours.
3	0.00002 cc.	.....	+ 24 hours.
4	0.000002 cc.	+ 60 hours.	+ 66 hours.

Here again the lack of protective power of W—'s serum for the heterologous strain C—, parallels its inactivity in vitro.

No.	Dose of Culture W—.	Series A. Normal Serum.	Series B. W— Serum, 5-28.	Series C. W— Serum, 5-31.	Series D. W—Ser., 6-10.
1	0.02 cc.	+ 40 hrs. cult. +	+ 40 hrs. cult. +	Lived.	Lived.
2	0.002 cc.	+ 48 hrs.	+ 66 hrs. cult. +	Lived.	Lived.
3	0.0002 cc.	+ 40 hrs. cult. +	+ 4 days cult. +	+ 6 days cult. 0.	+ 6 d'ys cul. 0.
4	0.00002 cc.	+ 4 days cult. +	+ 4 days cult. +	+ 6 days cult. 0. (Pericardial hemorrhage).	Lived.
5	0.000002 cc.	+ 5 days cult. +	+ 40 hrs. cult. +	Lived.	+ 6 d'ys cul. 0.

There was very efficient protective power in the serum of W— obtained at, and again ten days after the crisis, toward the homologous strain, whereas the serum three days before the crisis was no more active than was normal serum. This is parallel with the phagocytic activity of the heated serum.

To determine the parallelism of the phagocytic action of the sera in vivo and in vitro, studies were made of the sera of R— and of W—, which had shown the most marked protective power. Each of a series of mice were injected intraperitoneally with 0.2 cc. of the serum of R— obtained after lysis, and each of a control series with the same quantity of normal serum. Three hours later 0.02 cc. of culture R— was injected intraperitoneally in each mouse. Specimens of the exudate were removed at intervals with a capillary pipette, and stained with methylene blue. In the control animals receiving normal serum there was unrestricted multiplication of the organisms, which remained exclusively extra-cellular, and after one and two hours were present in the exudate in enormous numbers. In the protected animals there was no such multiplication evident. (The total number of organisms introduced in this dose is relatively small, and one must search very carefully to find any organisms.) After one-half, and one hour nearly all (though not quite all) the extra-cellular cocci had disappeared from the exudate and scattered leucocytes were seen containing one or more pair of ingested cocci. These were more numerous (as shown by Ungermann[35]) in smears made directly from the peritoneal surfaces. There was no evidence of extra-cellular lysis.

More definite pictures were obtained by the use of W— serum and culture. All the mice were prepared by injecting 1 cc. of sterile broth intraperitoneally 24 hours before the test. Mixtures were prepared, each containing 0.2 cc. of patient's serum and 0.2 cc. of a broth culture of the pneumococcus W—, and were injected into the peritoneal cavity of a series of mice, a second series receiving the same quantities of broth culture and normal serum. Specimens were removed with a capillary pipette at fifteen minute intervals.

After fifteen minutes in the normal serum controls the exudate contained many diplococci, all extra-cellular, and there was no evidence of phagocytosis. After one hour these had increased greatly in number. In the protected (posteritical serum) animals, even after fifteen minutes, the great majority of the extra-cellular organisms had disappeared, though a few scattered ones could be found until after the lapse of an hour. After fifteen minutes a considerable number of cocci were found inside the leucocytes, though the number found did not seem to account for all that had disappeared. After one-half hour there were about the same number of ingested cocci to be seen. After one hour but few were left, even in smears made directly from the peritoneal surfaces. This disappearance, in large part at least, is due to intracellular digestion. Even after fifteen minutes some of the ingested cocci stain poorly, and show evidences of solution. The same process is seen when the serum mixtures are injected into untreated mice, except that it takes place more slowly. There was no evidence of extra-cellular lysis.

In all of the four sera examined the activity was retained after heating one-half hour at 56° C. In some cases it was somewhat reduced. If the heating was prolonged, or if it much exceeded 56° C., the activity was destroyed. It persisted for several weeks in serum stored on ice, but gradually weakened, and finally disappeared. Except in one case dilution with salt solution destroyed its activity. The activity could never be increased or restored by adding fresh normal serum. The substances are, therefore, tropines, rather than immune opsonines. They are removed from the serum by digestion with the organism on which they act.

The strict limitation of the activity of each serum to the homologous strain is surprising, in view of the findings of Neufeld and of Cole, that most strains can be classified in a small number of groups. The explanation probably is that each strain differs to a certain extent from all, or most, other strains in its serum reactions. A serum as feeble as are the patient's sera, can show activity only under most favorable conditions; *i. e.,* when tested with the homologous strain of pneumomoccus. But a more potent immune serum, even though univalent, might act about equally on all those strains most closely related to the one used in producing the immune serum. The slight differences between the strains would thus be obscured and they would all fall apparently into a single group. Neufeld's work[7] indicates that this grouping of strains of pneumococci is not absolute, but that a very potent univalent serum while largely limited in its action to members of the corresponding group, may exert a certain amount of "overlapping" activity on members of other groups. It is interesting that none of the last three active sera tested showed any action on Neufeld's strain PnI, kindly furnished by Dr. Dochez as a typical example of the commonest group.

Of the sixteen cases, one has been omitted from the summary because it was tested only with an heterologous stock culture. A second one was also omitted because the strain isolated was so feebly virulent for mice that no definite conclusions as to the protective power of the corresponding serum could be drawn.

The results of these experiments may be summarized as follows:

Of twelve cases, in which satisfactory protection tests were carried out with the sera of patients after crisis, or lysis, using the homologous strain of pneumococcus, nine had sera which showed definite protective power for mice as compared with normal serum. The sera of three gave negative results; in these three cases but one specimen of serum was tested.

In two of the cases, the serum of which showed protective power after the crisis, specimens of serum obtained during the acute stage of the disease showed no such power. Further in two other cases, one fatal, in which the serum was examined only during the acute stage, no protection was manifested.

Three sera, which protected mice from the homologous strain, were also tested with an heterologous strain, and showed no protective power toward that strain.

Occasionally protective power cannot be demonstrated unless the serum is tested at frequent intervals during convalescence.

With but two exceptions, all the strains isolated were not phagocytable in fresh normal human serum, and were presumably virulent for man. The other two strains, which seemed identical with one another in every respect, were highly virulent for mice. As one was cultivated from the blood of a patient a few days before death, it seems most reasonable to regard it as also virulent for man, in spite of its phagocytability in normal human serum. This suggests the advisability of greater caution in assuming that the virulence of an organism for man is always parallel with its resistance to phagocytosis in the test tube.

Of eleven cases in which the phagocytic activity of the serum after crisis, or lysis, was tested in vitro, six showed definite activity. In five, negative results were obtained. In some of these, at least, positive results would probably have been obtained had suitable variations in technic been employed.

This activity has a significance which is quite different from that of a rise in opsonic index, which has often been described, but is qualitatively comparable with the activity of potent immune serum, in that it brings about *active phagocytosis of a virulent pneumococcus, not phagocytable in normal human serum.*

In two of these cases serum examined before the crisis showed no such activity. The serum of a fatal case was also inactive.

This phagocytic activity, with one exception was *strictly limited to the homologous strain,* derived from the patient whose serum was being tested.

The active substances in the serum are bacteriotropines, in the sense of Neufeld, since they resist heating at 56° C., and persist in the serum in vitro, for a considerable period.

In both of two cases tested, phagocytosis in the peritoneum of the mouse closely paralleled phagocytosis in the test tube. Intracellular digestion of the cocci, as shown by changes in staining reaction, was marked in both cases.

The phagocytic activity of the serum ran closely parallel with its protective power for mice, both in incidence, in time of appearance, and in strict specificity as to the strain of pneumococcus affected. It, therefore, seems justifiable to conclude that the protective action of the serum depends on, and is due directly to, its power of promoting phagocytosis.

A much larger number of cases must be studied and the constancy of the process demonstrated before drawing final conclusions as to the rôle it plays in man. But the fact that such definite phagocytic activity can be shown to develop at the crisis (or lysis) in at least a considerable proportion of cases, and that this activity is directed against, and largely limited to, the one virulent strain infecting the patient, makes it seem probable that this factor plays an important part in bringing about recovery in man.

In conclusion, I wish to express my thanks to Dr. Lewellys F. Barker for permission to study and report these cases, and also Dr. R. H. Major, Dr. T. P. Sprunt, and especially Dr. J. A. Luetscher for suggestions and assistance in carrying out the work.

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LECTURES ON THE HERTER FOUNDATION.

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LECTURE III.[1]

(With 22 text-figures.)

PIROPLASMOSIS.

The diseases included under the general term of piroplasmosis are amongst the most devastating which affect domesticated animals, and they are, consequently, of great economic importance. As far as known, all forms of piroplasmosis are tick-transmitted.

DISEASES DUE TO PIROPLASMA (=BABESIA).

Strictly interpreted, the term "piroplasmosis" applies to diseases caused by intracorpuscular parasites belonging to the genus *Piroplasma* (= *Babesia*[2]), which possess certain definite characters, produce definite symptoms, and are communicable by blood inoculation.

The striking features which characterize *Piroplasma* are the following: On examining the infected blood corpuscles and enumerating them in accordance with the types of parasites they contain it will be found that 30 to 40 per cent contain *two piriform parasites joined at their tapering extremities*—this appearance is characteristic of what I regard as the genus *Pirolasma*, of which the species *canis, caballi, ovis, bovis* or *bigeminum*, and *divergens* may be taken as examples. Of the remaining infected corpuscles about 50-70 per cent contain single parasites of varying form, whilst a few (anywhere from 0.5 to 11 per cent) show characteristic dividing forms, whose significance I pointed out some years ago with Graham-Smith. *Piroplasma canis* and *P. pitheci* differ slightly from other piroplasms in that some infected corpuscles (about 1-4 per cent) contain more than two parasites (*i. e.*, 4-16), although in *P. bovis* four parasites are occasionally found within a corpuscle. The usual mode of multiplication of *Piroplasma* is shown in the accompanying figure (Fig. 1).

True piroplasmosis occurs in cattle, sheep, horses and dogs in many parts of the world. The parasites which cause the disease in each of these species of mammals are specific in respect to their pathogenic action: for instance, the parasite which occurs in the dog (*P. canis*) is only capable of setting up the disease in the dog. Parasites having the morphological characters of *Piroplasma* have been discovered in the monkey and rat (*P. pitheci, P. muris*), but the symptoms they produce and their mode of transmission are unknown.

In the domesticated animals above enumerated, the presence of piroplasms in the blood is accompanied by a definite train of symptoms following upon the animals being attacked by pathogenic ticks. Usually about 8-10 days after the animals have been attacked by the ticks they show high fever, loss of appetite, hæmoglobinuria, icterus, and a large number of the animals die in a few days, anywhere from 25-100 per cent succumbing to the infection. The hæmoglobinuria is chiefly due to the destruction of the blood corpuscles by the parasites, the hæmoglobin being eliminated from the kidneys. The urine may appear lightly tinged with hæmoglobin or very dark, depending upon the intensity of the blood destruction. In severe cases the number of corpuscles in the circulating blood may be reduced to a third or less, the blood, consequently, appearing thin and watery. When animals "recover" they do so slowly, and the parasites grow very scarce in the blood, so that they cannot, as a rule, be detected microscopically.

The parasites persist in the blood of "recovered" animals for years after they have, to all outward appearances, resumed a healthy condition. "Recovered" or "salted" animals are not susceptible to reinfection, and consequently possess enhanced value in countries where piroplasmosis is endemic. The blood of "salted" dogs and cattle has been found to be infective for three to eight years after the acute attack has subsided. In nature, piroplasmosis is only transmitted by ticks which have previously fed upon infected animals, either in the acute stage of the disease or in the "salted" condition. It is owing to indigenous animals being "salted" in regions where piroplasmosis is endemic that the ticks of the region continue to harbor the parasite and constitute a potential source of danger to freshly imported animals coming from places where the disease is absent.

BOVINE PIROPLASMOSIS.

Bovine piroplasmosis is due to at least two species of *Piroplasma: P. bovis* and *P. divergens.*

The latter parasite (Fig. II), judging from specimens which have reached me hitherto, is confined to Europe and in nature is transmitted by *Ixodes ricinus* (Fig. III), the common European cattle tick. I have received specimens of *P. divergens* and *I. ricinus* from cattle suffering from "redwater" in Norway, Germany, Russia, Hungary, Great Britain, and Ireland, and I know that this form of redwater occurs in Finland, Sweden and France, where *I. ricinus* is prevalent. The transmission of the disease by means of larval stages of

[1] Owing to this lecture not having been written until after it was delivered in the Medical Department of the Johns Hopkins University, October 10, 1912, the author has taken the opportunity of adding a considerable amount of new matter and has somewhat altered the form of the lecture.

Figures I, IV, VI, XI, XII, XX-XXII are reprinted from *Parasitology*, Vols. I-V; figures III and V are reprinted from *Ticks*, Part 2; the remaining twelve figures (II, VII-X, XIII-XIX) have not hitherto been published. All except figure XI are by the author.

[2] The generic name *Babesia* has priority, and is coming into general use, although the name *Piroplasma* is more usually employed by American and British writers.

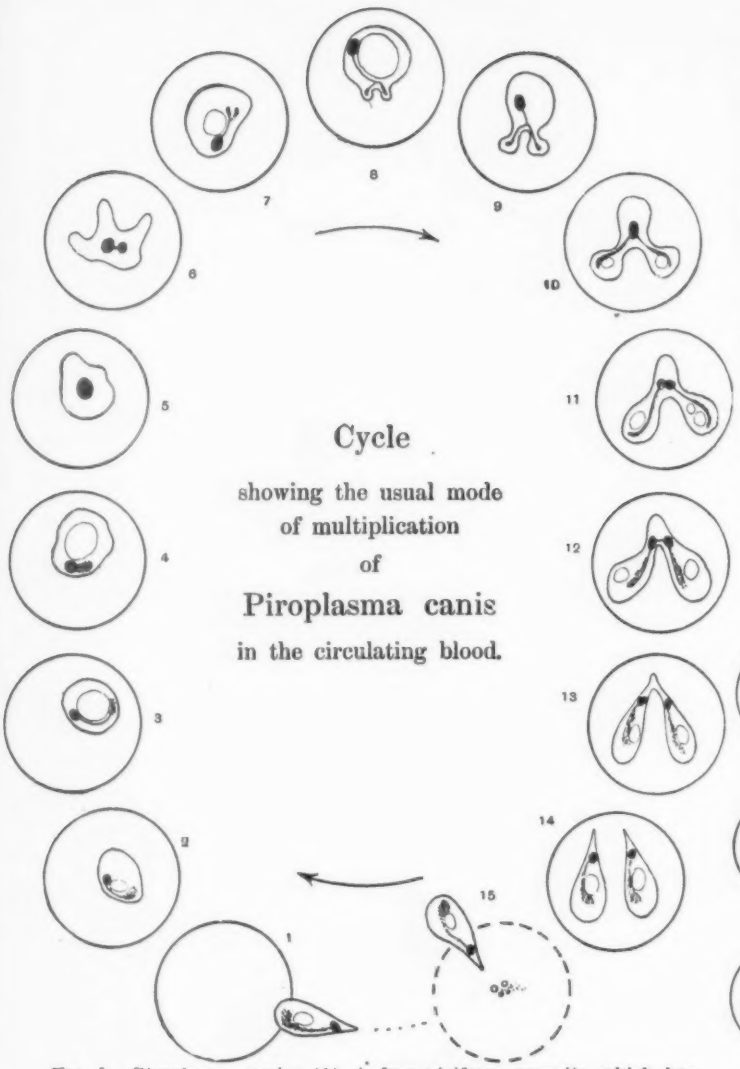

FIG. I.—*Piroplasma canis:* (1) A free piriform parasite which has just left a blood corpuscle enters a normal corpuscle and (2-4) assumes a rounded form, remaining quiescent for a time, after which it grows in size. It then becomes actively amœboid (5, 6), and again becomes rounded (7). Two symmetrical processes (8-10) are then protruded, which rapidly enlarge at the expense of the body of the parasite. Each of these processes (11-13) gives rise to a mature piriform parasite, which remains attached to its fellow by a thin strand of protoplasm. The parasites next become separated (14) and, by active swimming movements, burst out of the corpuscle (15) whose hæmoglobin escapes into the plasma. The free parasite immediately re-enters a fresh corpuscle. (Nuttall and Graham-Smith, 1907.)

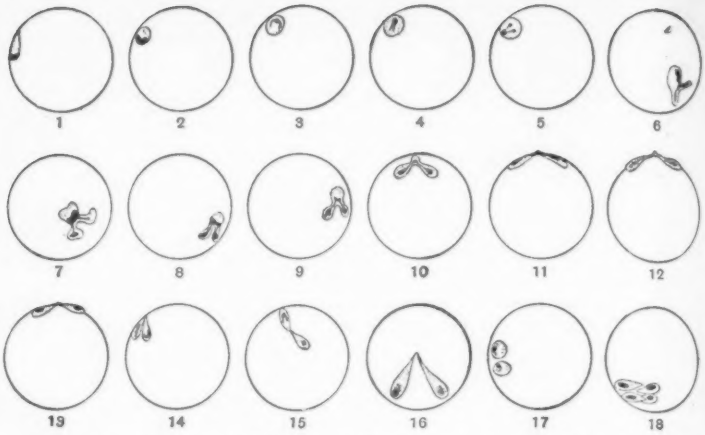

FIG. II.—*Piroplasma divergens:* Progressive stages of development corresponding to those observed in *P. bovis* (q. v.); the parasite is, however, much smaller than *P. bovis*, although occasionally fairly large piriforms (16) may be encountered. (Original, G. H. F. N. del.)

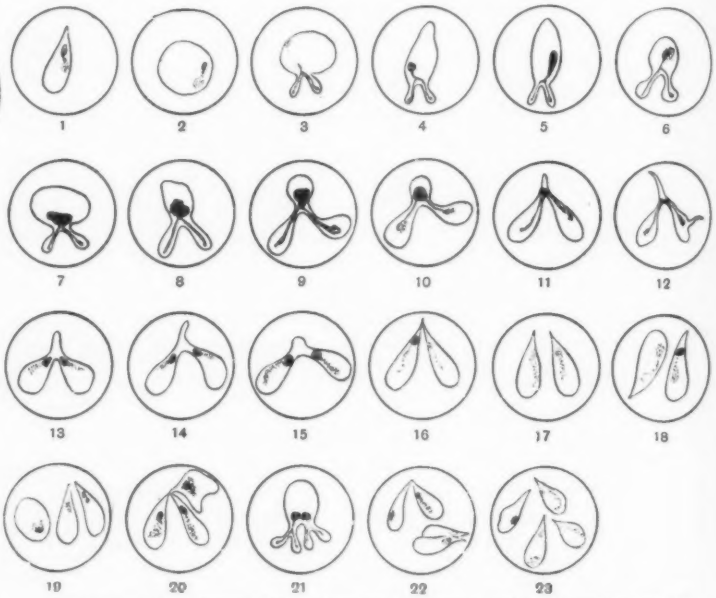

FIG. IV.—*Piroplasma bovis*, showing the same mode of multiplication as *P. canis;* (21) a parasite dividing directly into four. (Nuttall and Graham-Smith, 1907.)

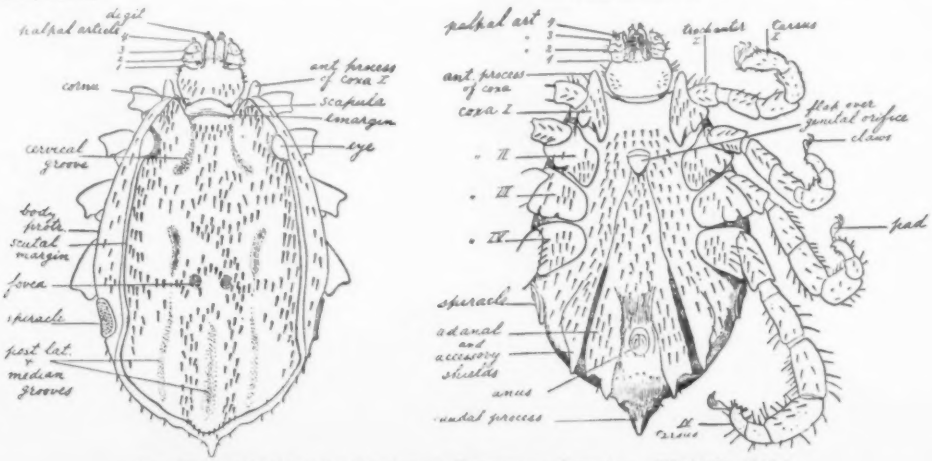

FIG. V.—*Boophilus decoloratus* ♂: Dorsum and venter. (Nuttall, 1909.)

I. ricinus, the progeny of ticks collected from diseased cattle suffering from piroplasmosis in Germany, England and Ireland, has been demonstrated experimentally by Kossel and his colleagues, by Stockman, and by me, respectively. Although the symptoms due to *P. divergens* infection are similar to those produced by *P. bovis,* the disease appears to be milder. Animals which have "recovered" from *P. divergens* infection are susceptible to infection with *P. bovis.* The parasites, moreover, show distinct morphological differences which were recognized by Kossel and others, although it is but recently that MacFadyean and Stockman gave *P. divergens* its distinctive name.

The well-known Texas fever of the United States, generally known as redwater or bovine hæmoglobinuria in other parts of the world, is due to *P. bovis* (Fig. IV) and appears to be transmitted in nature almost solely by *Boophilus annulatus* and its varieties, *B. australis* and *B. decoloratus* (Fig. V).

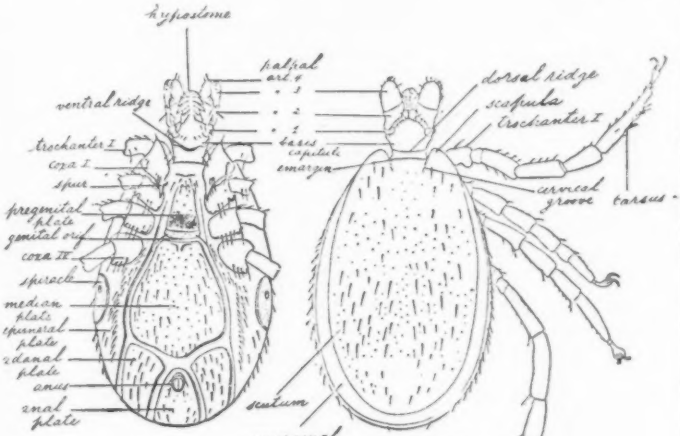

FIG. III.—*Ixodes ricinus* ♂, venter and dorsum. (Nuttall, 1908.)

Piroplasma bovis is widely distributed in warm countries and the tropics; it occurs in North and South America, Australia, Asia and Africa, and occasionally in Southern Europe. Redwater has been repeatedly reproduced experimentally by means of larval *Boophilus,* the progeny of ticks which have sucked the blood of animals suffering from redwater. This form of piroplasmosis has been the cause of great financial losses, it having been estimated that in the United States alone the annual loss has amounted to forty million dollars.

EQUINE PIROPLASMOSIS.

Redwater, or "biliary fever," in horses, as I have shown with Strickland, is due to two parasites which are morphologically distinct: *Nuttallia equi* (Laveran), of which I shall speak presently, and *Piroplasma caballi* Nuttall (Fig. VI), the cause of true piroplasmosis in equines. When a horse "recovers" from *P. caballi* infection it is still susceptible to *N. equi* infection. *Piroplasma caballi* occurs in Russia, Roumania, in Transcaucasia, and apparently extends across Siberia. *Dermacentor reticulatus* has been shown by experiment to transmit the disease, and this tick is distributed over the geographical area mentioned, besides occurring in Western Europe, including Great Britain, where it is, however, relatively rare. Although I have received blood-films from horses suffering from biliary fever in other parts of the world, none of the films contained parasites similar to *P. caballi.*[3]

OVINE PIROPLASMOSIS.

True piroplasmosis, due to *P. ovis,* has been observed in sheep in Italy, Turkey, Roumania and Transcaucasia. I have been informed that it has recently been discovered in Egypt.[4] The recorded presence of ovine piroplasmosis in South Africa and North America is due to errors of observation. It has been demonstrated by experiment that *Rhipicephalus bursa* conveys the disease. The geographical distribution of this tick, judging from specimens which have reached me, appears to be limited chiefly to Southern Europe, the Islands in the Mediterranean, Transcaucasia, and North Africa, although

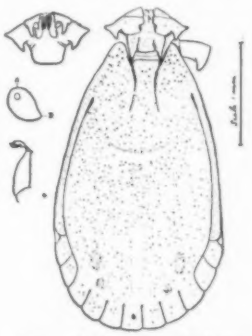

FIG. VII.—*Haemaphysalis leachi* ♂. (Original, G. H. F. N. del.)

Neumann states that it occurs both in East and West Africa as far as the Cape and also in the West Indies. I have only seen *P. ovis* in blood-films from Roumania and Italy.

CANINE PIROPLASMOSIS.

Two forms of piroplasmosis have been observed in dogs, the commoner and more widely distributed being due to *P. canis.* In India *P. canis* (Fig. I) is transmitted by *Rhipicephalus sanguineus,* a tick which has accompanied the dog practically all over the world. This tick is the probable vector of *P. canis* throughout Asia, Southern Europe and North Africa. *Haemaphysalis leachi* (Figs. VII-X), on the other

[3] On 17 January, 1913, Dr. S. T. Darling, Chief of the Department of Sanitation, Ancon, Panama, sent me a blood-film showing *P. caballi,* taken from a horse in Panama. The ticks he sent us for determination, and which were taken from the horse, were *Dermacentor nitens* and *Amblyomma cajennense*—it is probable that the former is the carrier, since it is usually found on horses.

[4] In blood-films received, 5 VI, 1913, from Mr. F. E. Mason, M. R. C. V. S. of Cairo, we were only able to detect a few small single intracorpuscular parasites, none of which were typical of true piroplasms.

hand, is the vector over the greater part of Africa from Cape Colony northwards. Attempts to infect the fox and jackal with the parasite have been attended by failure.

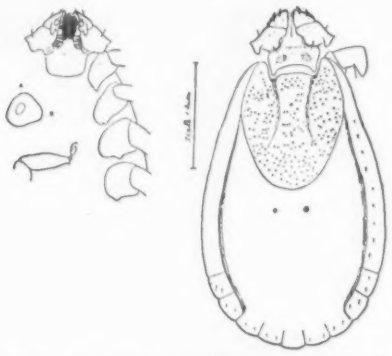

FIG. VIII.—*Haemaphysalis leachi* ♀. (Original, G. H. F. N. del.)

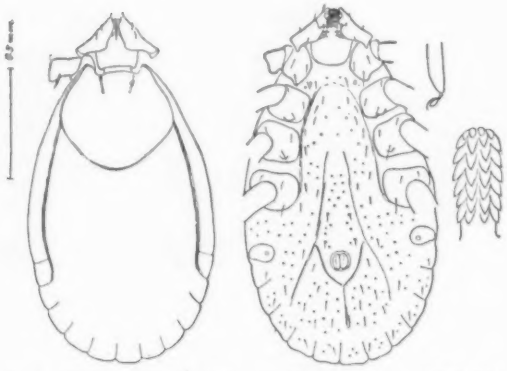

FIG. IX.—*Haemaphysalis leachi* nymph. (Original, G. H. F. N. del.)

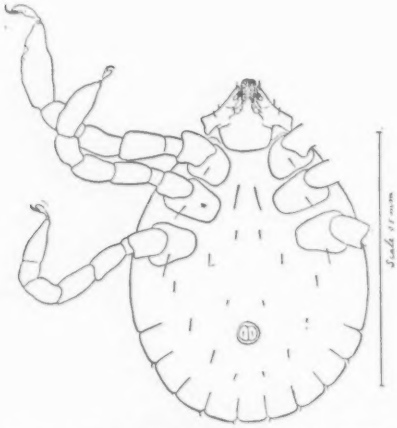

FIG. X.—*Haemaphysalis leachi* larva. (Original, G. H. F. N. del.)

On the other hand, another parasite (*P. gibsoni* Patton), which is somewhat different to *P. canis* morphologically, is stated to produce infection both in dogs and jackals in India.

CONCERNING THE TICKS WHICH TRANSMIT PIROPLASMA.

As I have already stated, piroplasmosis is conveyed to cattle by *Boophilus* and *Ixodes ricinus,* to sheep by *Rhipicephalus bursa,* to dogs by *Rhipicephalus sanguineus* and *Haemaphysalis leachi,* and to horses by *Dermacentor reticulatus.* A number of other species of ticks may, no doubt, play a part as carriers, but the ones I have named are the chief vectors, and in each case they have been proved to transmit piroplasmosis under experimental conditions.

Since piroplasmosis only occurs in the presence of ticks, it is now generally recognized that to grapple with the problem of prevention a knowledge of their life habits is essential. At the time at my disposal I can only refer briefly to the subject. All Ixodid ticks lay eggs, whence they emerge as hexapod larvæ. As soon as their chitinous exoskeleton has hardened sufficiently, the larvæ may feed upon a vertebrate host. After feeding they remain quiescent for a period, after which they moult and emerge as nymphs. These feed again, undergo metamorphosis, and finally emerge as adult males or females. In the case of *Boophilus* we have a one-host tick, for it remains, as a rule, upon the host from the larval to the adult stage, moulting twice whilst remaining upon the host. In *R. bursa* we have a two-host tick, since it remains upon the host during its larval and nymphal stages, moulting once upon the host; it abandons the host as a fully-fed nymph, undergoes its metamorphosis upon the ground, and emerges as an adult, which has to seek a second host. All of the remaining pathogenic species of ticks above enumerated are three-host ticks, for they drop from the host when replete, both in the larval and nymphal stages, to undergo their metamorphosis upon the ground; in other words, the larva, nymph and adult have each to seek a host. In all of the ticks here mentioned the sexes copulate upon the host, and the replete fertilized females drop to the ground, where they seek shelter and oviposit. The number of eggs laid varies with the species and individual. Taking average figures from my raising notes:

Boophilus decoloratus lays	1200-4500 eggs
Ixodes ricinus lays	2400-3200 eggs
Rhipicephalus bursa lays	4900-6900 eggs
Rhipicephalus sanguineus lays	ca 2000-3000 eggs
Haemaphysalis leachi lays	2400-4800 eggs

The rate of metamorphosis varies considerably according to the temperature at which the ticks are maintained. In *Boophilus,* the life-cycle is rapidly completed, because the tick is incubated upon the warm-blooded host throughout its parasitic period, and it does not lose time in having to find a host after each ecdysis. In *Boophilus,* the cycle from egg to egg may last 120 days; in *R. bursa* it lasts somewhat longer; in *I. ricinus* it lasts 178 days, these being minimum periods observed under experimental conditions.

BEHAVIOR OF PIROPLASMA IN TICKS.

When a fertilized female tick has fed upon an infected animal the parasites she imbibes undergo development within her body. About the fourth or fifth day after the parasites have entered the tick's gut there appear free, club-shaped

bodies, which move about with vermiform movements and are encountered in the gonads. Their further development is obscure, but in infective ticks both Christophers (Fig. XI) and Marzinowsky have found minute bodies, which may provisionally be called Sporozoites, in the salivary glands.

In *Boophilus* and *Ixodes* the larvæ, descended from infected parents, are infective. In *H. leachi* the larvæ and nymphs are not infective; the ticks, descended from an infected parent, only infect the host when they have attained maturity (Lounsbury, Nuttall). In *R. sanguineus* the larvæ are not infective, but the nymphs and adults are; when nymphs are fed on an infected host the adult tick is infective (Christophers). With *R. bursa* (two-host tick) the larvæ are not infective, but the adults are (Motas). I have found adults of *H. leachi* infective after starving for seven months, and believe that ticks harboring *Piroplasma* remain infective as long as they live.

Unfed ticks may withstand prolonged starvation. I have seen unfed larvæ of *I. ricinus* still lively after 176 days starvation; *B. annulatus* larvæ were lively after 251 days starvation;

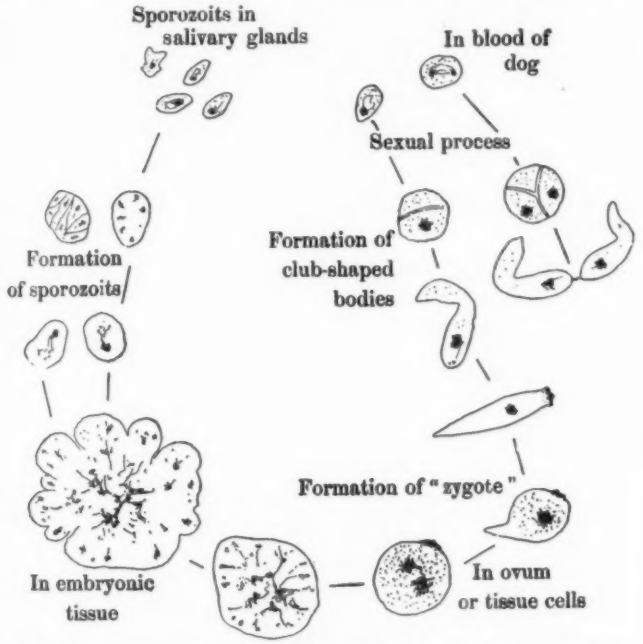

FIG. XI.—*Piroplasma canis*, showing the developmental cycle in *Rhipicephalus sanguineus* according to Christophers, 1912.

R. bursa adults were lively after 343 days starvation. In this connection I only mention the longevity of stages which may transmit piroplasmosis. It is clear that three-host ticks are the most difficult to eradicate by treating infested animals with tick-destroying dips or by removing the hosts from infested pastures for prolonged periods of time with the object of starving the ticks.

DISEASES DUE TO NUTTALLIA.

The first parasite belonging to this genus to be discovered was the one occurring in the horse to which Laveran (1901) gave the name of *Piroplasma equi*. As the morphology of this parasite does not agree with that of a true *Piroplasma*, as defined by me, França (1909) placed it in another genus which he named *Nuttallia*. That he was justified in taking this step is clear from subsequent observations carried out upon the parasite by myself and Strickland in 1910-1912 (Fig. XII), in the course of which we clearly differentiated it from *P. caballi*. Through the courtesy of Professor E. J. Marzinowsky, of Moscow, Russia, I have been able to examine blood-films showing so-called piroplasms in deer and reindeer, and I can also refer these to the genus *Nuttallia*.

Nuttallia equi appears to be much more widely distributed than *Piroplasma caballi* (q. v.); it produces a similar disease with hæmoglobinuria and jaundice, etc., in Italy, Sardinia, in

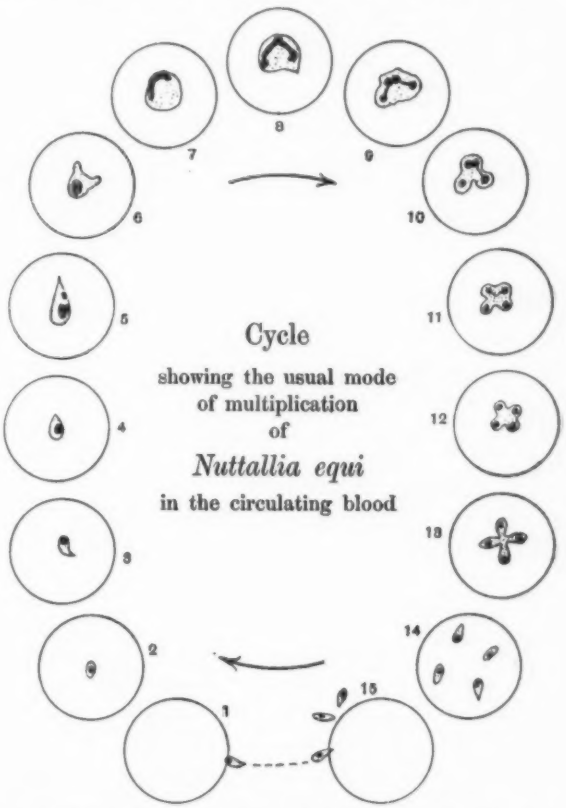

FIG. XII.—*Nuttallia equi:* (1) Entrance of minute oat-shaped parasite into a corpuscle; (2-5) the parasite grows in size whilst continually but slowly altering its shape; (6) larger parasite which is actively amœboid; (7-10) successive stages of division of the chromatin; (11-15) process of division repeatedly observed in living parasites: the formation and breaking-up of the cross-forms, the scattering of the daughter-cells within the corpuscle, and their escape from the corpuscle. (Nuttall and Strickland, 1912.)

many parts of Africa, Transcaucasia, India, Southern Annam and Brazil, from all of which countries blood-films have been sent to me for examination by correspondents.

Our experiments showed that a horse, after recovery from *P. caballi* infection, succumbed to the inoculation of *N. equi* blood. Conjoined pairs of piriform parasites are never observable in *N. equi* infection; about 90 per cent of the in-

fected corpuscles contain single parasites of all shapes and sizes, many of them being much smaller than any true piroplasm, 2-5 per cent of the corpuscles contain 2 to 4 parasites, and 1-5 per cent contain dividing, or "cross-forms." The accompanying diagram represents our conclusions as to the usual mode of multiplication of *N. equi* in the circulating blood. We studied the parasite in the living condition as well as in stained films.

Nuttalliosis resembles piroplasmosis in its symptomatology and pathology. I have a "salted" horse whose blood was virulent when last tested, a period of three years having elapsed since it was first infected. In Africa, as first shown by Theiler, the parasite is transmitted by *Rhipicephalus evertsi*. This tick, when fed upon infected horses in its larval and nymphal stages, is infective when adult. We do not know what species of tick serves to carry the parasites in other parts of the globe.

East Coast Fever of Cattle.

This devastating disease, which appears to be confined to Africa, is ushered in by the onset of fever following upon an

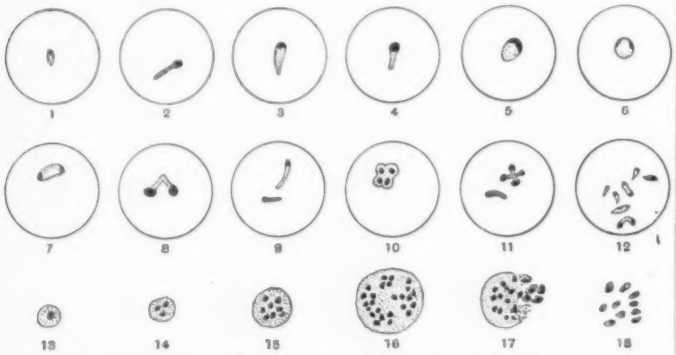

Fig. XIII.—*Theileria parva:* (1-12) stained intracorpuscular parasites; the figures arranged arbitrarily in a manner which would appear to indicate that they may divide into two or four parasites at a time, although division has not been observed to take place in living parasites; (13-18) Koch's bodies, a series of stained parasites from a spleen smear, likewise arranged arbitrarily in sequence to indicate their probable mode of development from uninellular bodies; (17 and 18) represent the breaking-up of the Koch's body and the liberation of its component elements. The black denotes chromatin, the stippling denotes the blue-staining protoplasm (Giemsa stain). (Original, and from Nuttall and Fantham, 1910.)

incubation period of 10-20 days after the cattle have been attacked by the ticks which convey the parasite. The fever continues usually for about 12 days, death taking place 18-34 days after the ticks have attacked the host. The characteristic symptoms of redwater, hæmoglobinuria, icterus and anæmia are absent in East Coast Fever. The mortality may amount to 80-90 per cent. Cattle which are immune to redwater are susceptible to East Coast Fever. In marked contrast to what is observed in redwater we find in East Coast Fever, as the disease progresses, that there is no appreciable decrease in the number of red blood corpuscles present in the peripheral circulation.

The cause of the disease, *Theileria parva* (Fig. XIII), differs in important respects from the parasites I have previously mentioned, and the disease cannot be communicated by the inoculation of blood containing the parasite, even when large quantities—up to seven litres—are injected.

Theileria parva is conveyed by *Rhipicephalus appendiculatus, R. simus, R. evertsi, R. nitens* and *R. capensis,* the first-named species of tick being the usual carrier. The parasites are not hereditarily transmitted in *Rhipicephalus,* but when taken up by the carrier at one stage of its development the tick is infective in the succeeding stage. When *R. appendiculatus* (Figs. XIV-XVII), for instance, sucks *Theileria*-infected blood as a larva the tick is infective as a nymph, or, having sucked infected blood as a nymph, it is infective as an

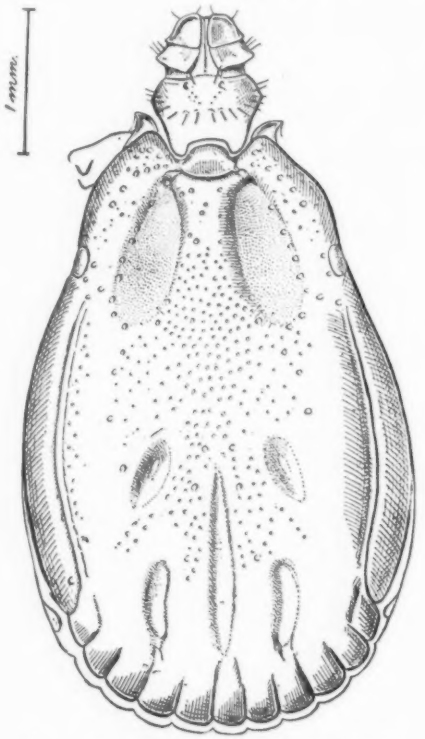

Fig. XIV.—*Rhipicephalus appendiculatus* ♂. (Nuttall, 1913.)

adult. Whereas, in redwater the parasites persist in the blood for years after recovery, and "salted" animals are capable of infecting ticks, the contrary holds for *T. parva, i. e.,* when animals recover from East Coast Fever they are incapable of infecting the ticks.

The parasites are at first present in small numbers, but 5-6 per cent of the corpuscles being invaded. The number of parasites, as a rule, steadily increases until death, when 60-75 per cent of the corpuscles in the peripheral blood are found to be infected. We have never observed multiplication of the living parasites in corpuscles, but we have seen them in a few rare instances escape from the corpuscles into the plasma. The parasites move about actively within the corpuscles. Whilst the parasite is very pleomorphic, the commonest forms

seen in stained preparations are ovoid or rounded and comma-shaped or clubbed. A proportion of the parasites is bacilliform. The appearance of the chromatin in some parasites suggests that multiplication may occur within the infected corpuscles, some of which contain up to eight distinct parasites. If, however, multiplication occurs within the corpuscles it must take place very slowly or we should have observed it in the living parasite. It will be of interest to determine, if *Theileria* multiplies in cultures as do malarial parasites and *Piroplasma canis*.

The results of our investigations forced me to the conclusion that the corpuscles merely serve as vehicles for the parasites, wherein they are housed and maintained until they reach

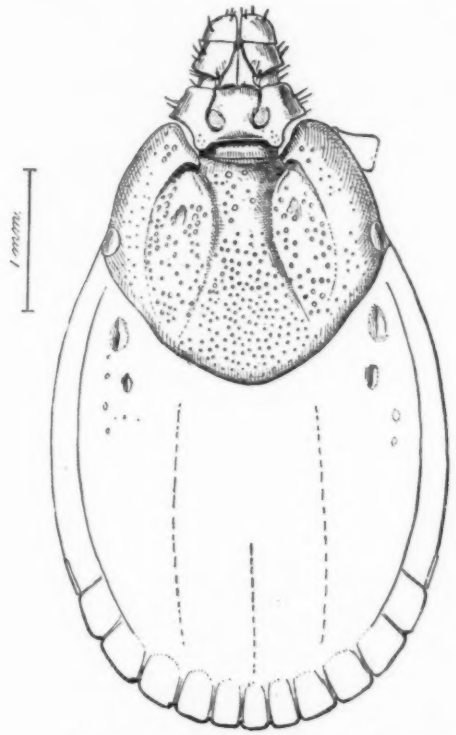

Fig. XV.—*Rhipicephalus appendiculatus* ♀. (Nuttall, 1913.)

their destination within the tick, which serves as their vector. The seat of multiplication and invasion of corpuscles appeared to me to lie in the internal organs, a view which subsequently received support from the investigations of Meyer, who communicated the disease by the intraperitoneal transplantation of large pieces of infected spleen.

Another striking feature connected with these parasites is the occurrence of "Koch's blue bodies" in the internal organs and occasionally in the peripheral blood. These bodies (Fig. XIII, 13-18) were first observed by Koch and to-day are considered to be of prime diagnostic importance. They are termed "blue" because of their appearance in blood-films stained by any of the modifications of the Romanowsky method. When examined stained they usually appear rounded, the blue-staining protoplasm containing discrete chromatin masses in varying numbers; at times they are seen to be breaking up, each mass of chromatin being accompanied or surrounded by blue-staining protoplasm. These bodies, which were regarded as developmental forms by Koch, have been made the subject of detailed study by Gonder, who states that he has seen them break up into their elements and scatter. They are encountered in the internal organs (lymphatic glands, spleen, etc.) before they appear in the corpuscles of the peripheral circulation. In some cases of East Coast Fever the parasites do not appear in the corpuscles, whilst Koch's bodies only are found in the internal organs. The bodies are found either free in the plasma or in cells—chiefly in lymphocytes—exceptionally in leucocytes. According to Gonder these bodies (which he terms "agamonts") undergo multiplication by schizogony, and I am inclined to agree with him. According to this view the parasites are first uninuclear; they grow, and the nucleus divides repeatedly until many

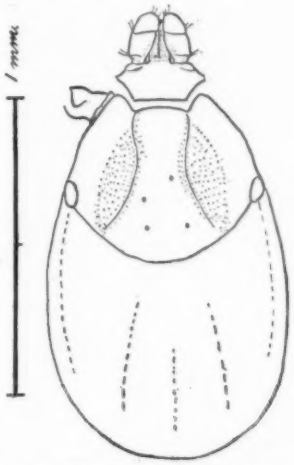

Fig. XVI.—*Rhipicephalus appendiculatus* nymph. (Nuttall, 1913.)

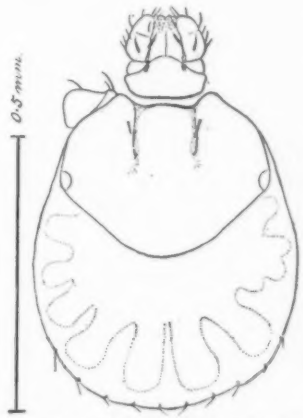

Fig. XVII.—*Rhipicephalus appendiculatus* larva. (Nuttall, 1913.)

small nuclei are formed, after which the minute parasites separate and scatter. It is presumably these small parasites which invade the corpuscles. The rest of the cycle of development, as outlined by Gonder, is so purely conjectural that we can afford to ignore it for the present. It is reasonable to assume, from what I have previously stated, that some of the intracorpuscular parasites are sexual forms of the parasites destined to undergo further development in ticks. That such a development occurs still remains to be determined, although Gonder believes that he has partially traced it.

Remarks upon some other parasites which appear related to the foregoing.

In the foregoing instances we have dealt mainly with three distinct forms of intracorpuscular parasites whose mode of transmission, through the agency of ticks, has been demonstrated repeatedly by experiments conducted upon cattle, horses, sheep and dogs. All of these parasites have until

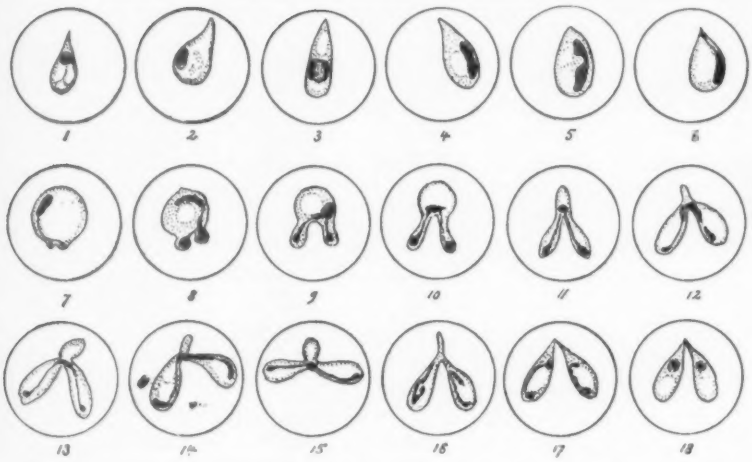

FIG. VI.—*Piroplasma caballi*, showing the same mode of multiplication as *P. canis*. (Nuttall and Strickland, 1912.)

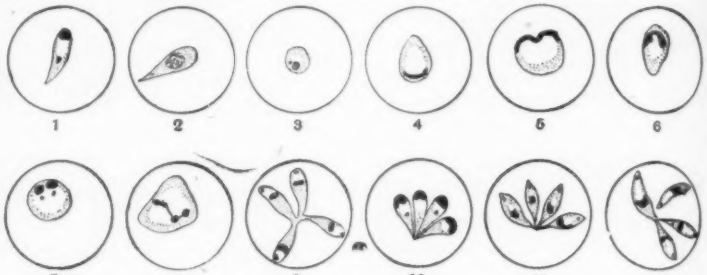

FIG. XIX.—*Nicollia quadrigemina* (Nicolle, 1907): Different stages drawn from a blood-film kindly lent by Professor C. Nicolle. (Original, G. H. F. N. del.)

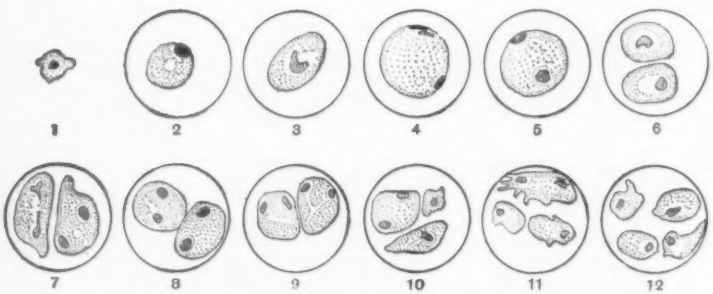

FIG. XVIII.—*Rossiella rossi* (Nuttall, 1910): (1) Single free parasite; (2-3) single uninucleate intracorpuscular parasites; (4-12) progressive stages of division into four parasites.

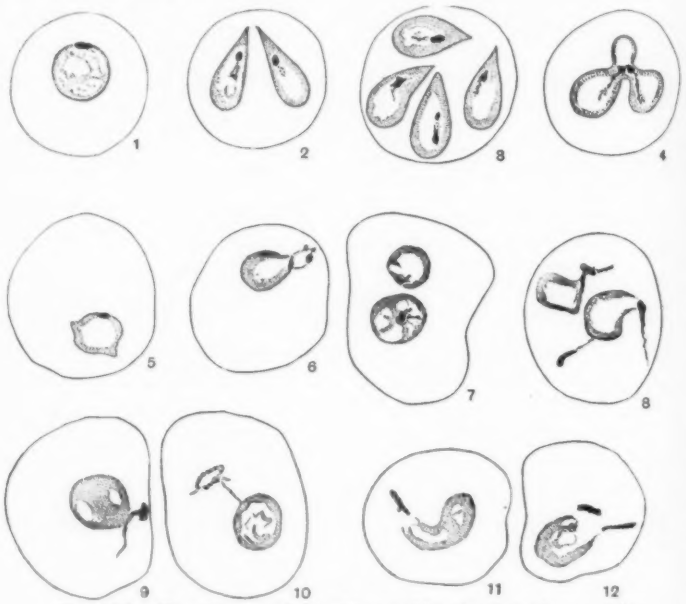

FIG. XX.—Showing the effect of trypanblue treatment upon *Piroplasma canis* in the peripheral blood of a dog; (1-4) normal types of parasites; (5-12) parasites from the same dog, showing progressive degrees of degeneration in blood removed from the dog's ear-vein 6 hours after the injection of the drug. (Nuttall, 1910.)

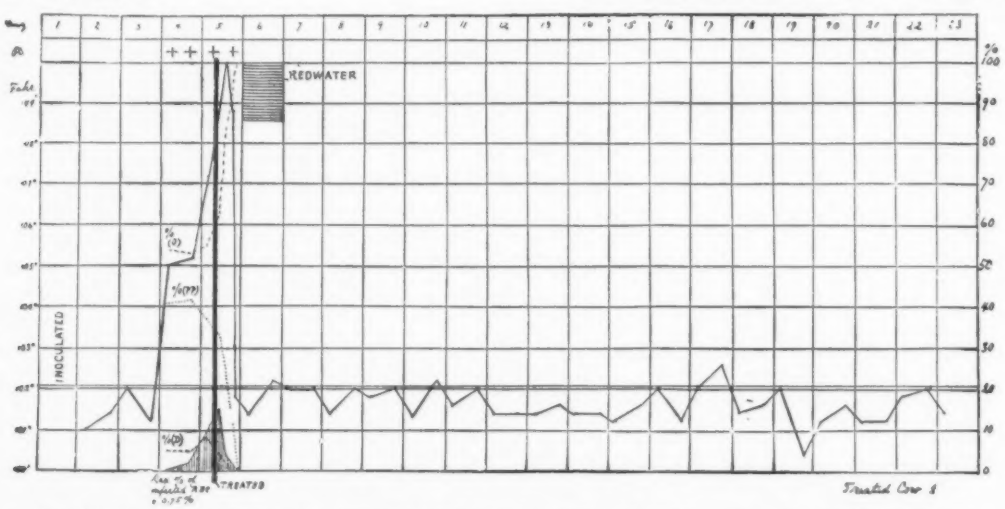

FIG. XXII.—Chart showing the course of events in a *trypanblue-treated cow*. See description of Fig. XXI. (Treated Cow I. Nuttall and Hadwen, 1909.)

recently been grouped in the one genus, together with certain others to which I shall refer more briefly. It has long been evident to me that the genus *Piroplasma*, or *Babesia*, was being regarded as a "hold-all" for parasites of very varied character, and it appears necessary to separate them more clearly. Dismissing, then, the three genera I have dealt with, viz., *Piroplasma*, *Nuttallia* and *Theileria*, we may consider the following:

"PIROPLASMA MUTANS" THEILER, 1907.

This parasite occurs in cattle in South Africa and Madagascar; it is very minute, resembles *Theileria parva* microscopically but for the absence of Koch's blue bodies, and is likewise transmitted by ticks. Contrary to *Theileria*, the parasite is, however, readily transmitted from animal to animal by blood inoculation, and is practically harmless in respect to pathogenic effects. The parasite is conveyed by *Rhipicephalus simus*, *R. evertsi*, and, rarely, by *R. appendiculatus*. When animals are inoculated a long period of incubation (60-115 days) elapses before the parasites appear in the blood. These data regarding *P. mutans* are based upon the confused accounts given by Theiler and a few statements made by Gonder. I have only studied the parasite in a few stained blood-films. It is clear that the parasite requires to be adequately studied before its position can be determined.

I would refer briefly, moreover, to the following parasites which have been observed in different animals but regarding which we require more information:

Rossiella rossi (Nuttall, 1910), found in the jackal (*Canis adustus*) in British East Africa. The parasite was referred provisionally by me to the genus *Piroplasma*, but subsequently to a separate genus (1912). The parasites (Fig. XVIII) are of large size, usually rounded, occurring singly, in pairs, and occasionally in fours. The nucleus of the parasite is very large and rounded when at rest. Multiplication occurs by direct division of the nuclei, two daughter parasites resulting, which may in turn subdivide in a similar manner.

Nicollia quadrigemina (Nicolle, 1907), found in the gondi (*Ctenodactylus gundi*)—a rodent—in Tunisia by Nicolle, and referred by him to the genus *Piroplasma;* but it differs markedly from the members of this or any other genus, and a new genus was therefore founded for it by me in 1908. The parasites (Fig. XIX) are oval or piriform, are commonly grouped in fours, often in a fan-like manner, and show distinct binuclearity.

I am not clear as to the position of a considerable number of parasites in different animals and which have been referred to the genera *Piroplasma*, *Theileria*, *Achromaticus* and *Microsoma* by various authors. These parasites require further study before they can be definitely classed.

TREATMENT AND PREVENTION.

In concluding, I must mention the subjects of treatment and prevention. The only drug hitherto discovered which exerts an influence on some of the parasites I have described is trypanblue,[5] a dye which is administered intravenously in preference to subcutaneously in 1-1.5 per cent aqueous solution. A dose of 5-10 cc. is curative for dogs suffering from *Piroplasma canis* infection, and the drug is being used to-day in many parts of Africa where it was previously impossible to keep dogs. Trypanblue exerts the same effect on cattle and horses suffering from *P. bovis* and *P. caballi* infection; it has proved of value in practice in the treatment of horses and, apparently, of cattle, the dose being 100-150 cc. of the solution.

Trypanblue has no effect upon the parasites of East Coast Fever, and I have no satisfactory records of its having been tried upon the other parasites I have described.

Under the influence of trypanblue (Fig. XX) *Piroplasma* rapidly degenerates and the parasites can no longer be discovered microscopically in the blood. The fever and hæmoglobinuria cease, and the animals recover from the other clinical manifestations of piroplasmosis. (Compare Figs. XXI and

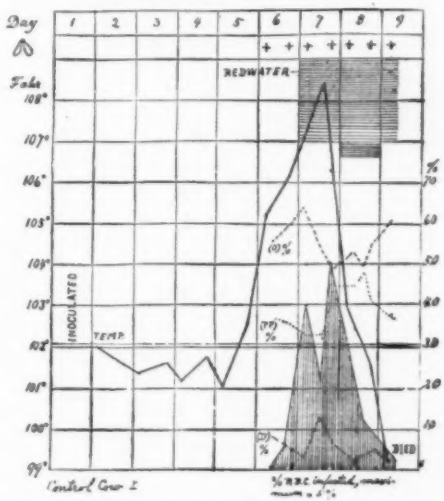

FIG. XXI.—Chart showing the course of events in an *untreated* cow which died of redwater on the morning of the 9th day after inoculation with virulent blood. The presence of parasites (microscopically) in the blood is indicated by + marks on days 6-9; the intensity of the hæmoglobinuria is indicated by the amount of horizontal shading above, whilst the vertical shading below indicates the percentage of *P. bovis*-infected corpuscles. (Control Cow I. Nuttall and Hadwen, 1909.)

XXII). The animals continue, however, to harbor the parasites in their blood for years, as can be shown by inoculation into clean animals; in other words, the animals recover in the vast majority of cases from the acute disease, and, especially in dogs, are "salted" like the animals that recover in nature. Whereas untreated dogs usually die, the treated animals usually recover.

I may note that the inoculation of cattle is commonly employed with the object of obtaining "salted" animals, which are, naturally, more valuable than the "unsalted," in that they resist reinfection when exposed in endemic areas.

The efforts at prevention have been directed against the

[5] Nuttall & Hadwen (1909).

ticks which transmit the parasites and their transportation from place to place by their hosts. They consist in the use of dips, most of which contain arsenic, in which the animals, especially cattle, are immersed at intervals of a few days or a week or more. Where there are no forests to be endangered, the burning of the dry surface vegetation has been found of some use in destroying the ticks which infest the pasture lands, and, finally, the removal of cattle for a period from infested pastures leading to the starving out of the ticks (especially *Boophilus*) has been found of use.

The preventive measures have to be based upon a knowledge of the life-histories of the ticks which transmit the parasites, and where they have been feasible and have been carried out intelligently they have been of great benefit, large tracts of country in the United States, Australia and Africa having been rendered almost tick-free by these measures.

ENTEROGENOUS MESENTERIC CYSTS.

By Robert T. Miller, Jr., M. D.,

Surgeon to St. Francis Hospital, Pittsburgh, Pa.

Until recent years the subject of mesenteric cysts has been notable chiefly for the obscurity surrounding it. There have been numerous contributions made within the past 13 years, however, and while our knowledge is still not comprehensive, yet to-day we possess much more information concerning these curious tumors than is generally appreciated. The historical aspect of the subject was well presented by Moynihan [1] in 1897. In 1900 Dowd [2] published a notable paper which merited the wide recognition accorded it; this article has been largely responsible for the active interest in the general subject shown in the past 12 years, for it was an original contribution, and, in addition, stimulated several investigators into decidedly productive efforts. Owing to the many different captions under which the subject is discussed, it is practically impossible to conclude a review of the literature with certainty of completeness; it will suffice perhaps to say that with the help of the Index Medicus and Hildebrandt [3] it has been possible to study more than 100 papers appearing since 1900; the most notable are those by Colmers,[4] Terrier and Lecene,[5] Lewis and Thyng,[6] Niosi [7] and Bauer.[8] A detailed presentation of a single case is of value, in view of the following facts concerning mesenteric cysts, viz: there is wide difference of opinion as to their genesis; a positive diagnosis has probably never been made before operation or autopsy; and their surgical significance is almost universally ignored. In a summary of 35 cases Colmers found a mortality of 35 per cent, and yet our text-books accord the condition very scant notice.

The following case was referred to the surgical clinic of St. Francis Hospital from the Department of Obstetrics of the University of Pittsburgh, by Professor Charles Ziegler:

Intestinal obstruction, due to a congenital enterogenous mesenteric cyst causing volvulus. Operation on the 4th day of life: resection. Death.

F. F. (No. 1493) female, 4 days old. Admitted September 28, 1912. Complaint: Vomiting and absolute constipation since birth. The patient is the last of thirteen children, all of whom, together with mother and father, are alive and well. Labor was neither prolonged nor difficult, no forceps being used. The baby has not retained any nourishment since birth; she has vomited everything taken, the vomitus being greenish-black in appearance. The bowels have not moved since birth. There has been no stain on the napkin.

Examination.—The baby is evidently exceedingly ill. When undisturbed she cries at intervals in a thin weak voice as though suffering paroxysms of pain. There is marked cyanosis of the face and hands. The skin is dry and wrinkled everywhere, but especially so over the extremities. The facial expression is pinched and drawn and the legs and arms are moved but little and that weakly; the picture suggests exhaustion due to suffering of some duration. The stump of the umbilical cord is still present with a small drop of purulent discharge at its base. The abdomen is symmetrical and not unusually distended. There is no visible peristalsis. Palpation causes the child to cry constantly and is unsatisfactory. Muscle spasm cannot be definitely recognized. No mass is palpated. The abdomen is generally tympanitic; there is no dullness in the flanks. On rectal examination the little finger can be inserted its full length and encounters no obstruction or mass. There is no blood or meconium on the examining finger. Temperature: 97° F. per rectum.

Diagnosis.—Intestinal obstruction, cause unknown.

As a long ambulance trip was possibly accountable in part for the child's poor condition, active efforts to raise her body temperature were made during the preparation for the operation.

Operation.—Under chloroform anæsthesia a right rectus incision was made. On opening the peritoneum there was seen both dilated and collapsed cyanotic loops of small bowel. Digital exploration revealed in the right flank just below the level of the umbilicus a freely movable mass which was easily brought up into the incision. This proved to be an intra-mesenteric tumor roughly oval, about 4 cm. long in its greatest diameter, and quite firm; over the dome of the mass and in very close relation to it there coursed a loop of small bowel, evidently about the level of the jejunum. This loop was flattened out into a thin and relatively wide band by the pressure of the subjacent tumor. There was a volvulus of the mass and affected sector of the bowel of rather more than 180 degrees in the direction of the hands of a clock, causing complete intestinal obstruction; the afferent bowel was greatly dilated and the efferent bowel quite collapsed. After reduction of the volvulus a circumferential area of pressure necrosis with a minute perforation was found on that portion of the afferent loop which had been drawn into the volvulus; in a limited area about the pressure ring the peritoneum was lusterless and in places there was a delicate deposit of fibrin. Cultures were taken from this area. The patient's condition urgently demanded haste, but a rapid enterostomy was not feasible since the obstruction was evidently high in the jejunum and drainage of the bowel at this point would have resulted in prompt starvation of a very weak patient. Immediate and permanant restoration of the continuity of the bowel seemed, however, imperative and accordingly a typical resection and lateral anastomosis were hurridly made and the abdomen closed. The baby's condition seemed about as good as before operation.

When put to the breast shortly after the operation the patient seemed too weak to nurse, and though she retained milk given with a medicine dropper, she grew gradually weaker and died five hours later.

From the autopsy notes it will suffice to abstract the following pertinent points:

On opening the peritoneum it was seen to contain a considerable amount of clotted blood, which was present in all parts of the abdominal cavity. No evidence of an inflammatory reaction on the peritoneum could be observed. The stomach was dilated by gas, but was otherwise empty. The duodenum and upper part of the jejunum were markedly distended and dark in color. The distension extended down to the point of anastomosis, viz.: about 10 cm. from the pylorus. The suture line in the bowel was intact, as the intestinal contents could not be squeezed out. Below this point the entire bowel was collapsed. The autopsy was quite negative aside from the above findings. *B. coli* grew in pure culture in the inoculations made from the region about the perforation of the bowel.

The tissue removed at operation proved of great interest. The specimen consists of a loop of small bowel approximately 8 cm. in length, within whose mesentery there is a mass, roughly egg-shaped, measuring 4 cm. in its long axis and 3 cm. in its transverse axis. The mass lies between the mesenteric leaflets in close relation to the bowel, but projects much more upon one mesenteric aspect than the other in such a manner as to cause the mesenteric leaflets to join in a line which runs diagonally across one lateral aspect of the mass, as shown in the illustration. Over the surface of the mass course many rather large vessels. For a distance of 4.5 cm. the bowel is greatly compressed by the subjacent mass being flattened out into a ribbon-like band 1.5 cm. across at its widest point. On the afferent loop at a distance of 1 cm. from the mass there is a necrotic area, which has divided the bowel completely, with the exception of the peritoneum, in one-half of the circumference. The afferent loop is greatly dilated, measuring 4 cm. in diameter; the efferent loop is collapsed, measuring .6 cm. in diameter. The mass is regular in outline and its surface smooth; it is uniform in consistency, rather hard, but definitely fluctuant. On section it is seen to be a simple cyst, from which escapes a whitish mucoid fluid. The wall of the cyst varies in thickness from 1.2 mm. to 2.7 mm. The interior presents a smooth regular surface, grayish-white in color, and in places showing delicate parallel ridges running in a plane at right angles to the long axis of the cyst. That portion of the bowel which courses over the tightly distended cyst has been so stretched in width that its lumen is represented by a mere slit, crescentic in form; in this region the wall of the bowel and the wall of the cyst are blended into one structure which forms a partition between the two lumina. There is no communication between the bowel and the cyst.

Sections cut from the cyst wall at various points show a very regular histological arrangement. The cyst is lined by a single layer of epithelial cells resting upon a tissue layer analogous to submucosa; the next layer consists of smooth muscle whose internal half is cut in cross sections and whose external half is cut longitudinally. Beneath the peritoneum there is at places a layer consisting of many fine blood vessels held in a loose connective tissue stroma.

The lining epithelium consists of a single layer of high columnar cells whose protoplasm stains fairly well and whose nuclei are arranged uniformly at the base of the cells. Over the major portion of the cyst wall these cells are set closely together and thrown up into well-developed villi which have a connective tissue stroma and contain uniformly a capillary vessel. The villi reach their maximum size and frequency at a point on the cyst wall quite distant from the bowel; as the bowel wall is approached they gradually decrease in height and number and finally disappear, while the epithelial cells, markedly decreased in size, assume the low columnar type, which in turn changes to the cuboidal type just before the bowel wall is reached. Over that portion of the cyst wall subjacent to the lumen of the bowel the epithelium is quite flattened out. An occasional goblet cell is encountered. No ciliated cells are seen. Nasse[9] reports almost the same epithelial arrangement in an enterogenous mesenteric cyst excised on the 6th day of life under circumstances quite similar to those in my case.

Beneath the epithelium is a layer of loosely arranged connective tissue stroma enclosing blood vessels and numerous gland-like structures whose tubules are lined by epithelium quite similar to that on the surface. This layer, which resembles the submucosa, is well marked in the region where the villi are best developed, but gradually thins out as the bowel wall is approached; directly subjacent to the lumen of the bowel this layer is represented merely by a few connective tissue fibers. Throughout the entire cyst wall the two muscle layers are well developed and sharply differentiated, the one always being arranged at right angles to the other. As the bowel is approached the inner layer splits into two portions which ensheathe the bowel and are continuous with its inner circular muscle layer. The external layer of muscle in the cyst wall remains intact and is directly continuous with the external longitudinal muscle of the bowel, thus forming a muscular envelope common to them both.

Apart from its structure as described above the bowel presents the usual appearance of the upper jejunum, viz.: numerous well-developed villi, with high columnar epithelium, and many goblet cells resting upon a typical submucosa. However, the structure alters as the cyst wall is approached; the villi disappear rather abruptly, and the high columnar changes to cuboidal epithelium, which lines perhaps one-fifth of the circumference of the lumen, and rests upon a submucosa almost devoid of secreting glands.

With overdistension of the cyst its wall will give at the weakest point, viz.: that portion acting as a septum between the lumina of the bowel and the cyst, since here there are lacking the whole external and one-half of the internal muscular layers of the cyst wall. Stretching of this septum accounts satisfactorily for the tissue changes occurring in both the cyst and the bowel at this point.

To summarize: The specimen consists of an intra-mesenteric cyst whose wall is, in part, directly continuous with that of the jejunum and whose histological structure resembles closely that of the adjoining portion of the bowel. Its structure and arrangement point directly to an enterogenous origin by a process of sequestration during embryonic life.

The genesis of mesenteric cysts was a very obscure subject until rather recent years. The ideas of the older pathologists were indefinite; such cysts were attributed variously to lymph stasis, with resultant dilatation of a lymphatic gland or vessel, and to cystic degeneration of a lipoma or of tuberculous glands. Dowd's article in 1900 aroused renewed interest in this subject, and recent work has resulted in a very considerable increase in our knowledge, though even now there is not a general agreement of opinion among the different investigators in this field. For instance, Dowd believes all mesenteric cysts to be of embryonic origin, while Niosi, in an excellent paper, states his belief that about one-half of all mesenteric cysts are acquired and places the so-called lymphatic and chylous cysts in this group. Klemm,[10] on the other hand, finds the wall of lymphatic mesenteric cysts to be made up wholly of those cells which arise from the mesoderm, and argues that these cysts are neoplasms developing from misplaced or sequestrated portions of mesodermic tissue, and hence form a group of tumors of embryonic origin for which he proposes the name of mesodermoids by way of analogy to that group of tumors which are now called dermoids. Arguments based on cellular structure must take into account the possibility of histological alterations in the wall of a cyst, for such changes could readily

obscure the picture; inflammation can substitute scar tissue for any other tissue. Conclusions based upon the character of the cyst contents can hardly be considered final, for, as Dowd has suggested, the fluid that collects in a preformed cyst is probably a mere matter of chance, whether lymph, chyle or blood from a ruptured mural vessel.

The state of our knowledge up to 1897 is perhaps best represented by Moynihan's classification of mesenteric cysts which appeared in that year, viz: 1, serous; 2, chylous; 3, hydatid; 4, blood; 5, dermoid; 6, cystic malignant disease; be it noted that only one group in this list suggests an embryonic origin. In 1900 Dowd successfully removed from the transverse mesocolon of an adult woman a cyst which closely resembled in detail a multilocular ovarian cystoma. The ovaries of this patient seemed normal on palpation. In presenting this case Dowd pointed out the close anatomical relationship existing in embryonic life between the Wolffian body or germinal epithelium and the root of the mesentery, and suggested the possibility of the sequestration from the germinal epithelium of a group of cells which might be displaced by the subsequent growth of the individual in such a way as to take up an intramesenteric position; later on, perhaps during the adult life, such a sequestrum from the germinal epithelium might develop into a tumor similar to the one he removed. Going further, he suggests that small portions of the developing gut may be similarly sequestrated, to lie perhaps between the leaves of the mesentery and later develop into a cyst. He suggests as a classification of mesenteric cysts the following, viz: 1, embryonic; 2, hydatid; and, 3, cystic malignant disease. The emphasis he laid on the probable embryonic origin of a large number of mesenteric cysts represents a decided advance in our knowledge of this subject. Since Dowd's paper this idea has been repeadly emphasized and elaborated; in a lengthy study, in 1907, Niosi presented what is probably our best working classification of mesenteric cysts of embryonic origin, viz:

1. Cysts of intestinal origin:
   (a) By sequestration from the bowel occuring during development.
   (b) From Meckel's diverticulum when it arises from the concave side of the bowel [or acquires an intramesenteric position]. (The portion in brackets is added by the author.)
2. Dermoid cysts.
3. Cysts arising from retroperitoneal organs; viz: urogenital organs (germinal epithelium, ovary, Wolffian body, Mullerian duct).

The idea that a portion of an embryonic tissue or organ may become sequestrated and still continue to develop, though usually in an anomalous manner, is a familiar one. Bauer credits Verneuil and Remak with the first proposal of the idea, while quoting Ribbert's[11] expression of the theory as the best. A free rendering of this passage from Ribbert is perhaps not superfluous: "Epithelial cysts may be formed not only as a result of trauma, but also by abnormal development; irregular growth may cause a change of position or a changed relationship without actual change of position; or, again, epithelial structures which should coalesce fail to do so, or finally those which should decrease in size or disappear entirely retain their maximum dimensions. A group of epithelial cells with its connective tissue support is rendered more or less independent in growth by either of these processes and thenceforth develops as a cyst whose lumen is constantly enlarged by the retention of its own secretion and desquamated epithelium, although this increase in size is usually extremely slow and scarcely noticeable." When suggesting this process to explain the enterogenous origin of certain mesenteric cysts, Dowd argued by analogy, instancing the comparative frequency with which atypical development results in an accessory thyroid, spleen, or pancreas. The actual process of sequestration from the developing gut was not observed until 1908, when there appeared a report from Lewis and Thyng in which they describe the "regular occurrence of intestinal diverticula in embryos of the pig, rabbit and man." Previous to this time Lewis had discovered a "knob-like outpocketing of the intestinal epithelium a short distance beyond the pancreas" in a rabbit embryo, while Thyng had found a similar structure in a human embryo of 13.6 mm. and had interpreted it as an accessory pancreas. During further investigations, however, it was found that in the development of the small bowel of the embryos mentioned there always occurs a series of "knob-like intestinal diverticula," which appear earliest upon the proximal portion of the small bowel, together with the "cystic and bile ducts." In older embryos these structures were found in greater numbers on the distal portion of the small bowel; none were found on the large bowel, but the authors considered their presence here quite possible in more advanced embryos.

These diverticula consist of downgrowths of the intestinal epithelium which may terminate in the muscular coat of the bowel or may extend quite through the bowel wall to form knob-like projections. "They usually degenerate and disappear," but sometimes their continuity with the intestinal epithelium is destroyed and they persist as minute epithelial cysts or solid nests of epithelial cells lying in the muscle, or upon or near the bowel. One drawing shows such a cyst lying in the mesentery and in contact with the muscle of the duodenum in a pig embryo of 20 mm. Speaking in a later paper of the occurrence of duodenal diverticula in the adult, Lewis[12] suggests the possibility of their congenital origin, "although apparently no case has yet been recorded at birth," and, in discussing cysts, reference is again made to the embryonic mesenteric cyst just cited, with the suggestion that "it is possible that certain of the congenital intra-mesenteric cysts have a similar origin." The occurrence of these diverticula and cysts is not limited to the mesenteric border; when occurring on the convex border they exhibit changes in shape and size which suggest an alteration due to the rapid growth of this portion of the bowel. It may be that this relatively rapid growth causes the much more frequent disappearance of those so situated than of those lying in the mesentery.

Lewis and Thyng have thus established the fact that the formation of diverticula and cysts is a regular occurrence in the embryonic development of the gut. There can be scarcely

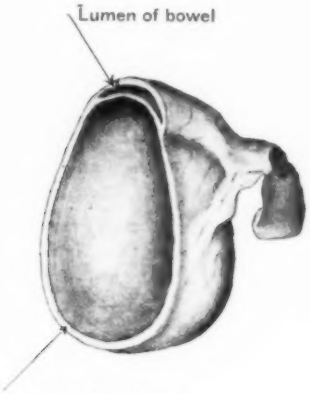

FIG. 1.—Cross section showing the compression of the bowel by the tense cyst.

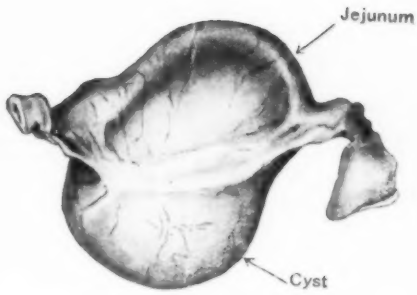

FIG. 2.—Cross section showing the compression of the bowel by the tense cyst.

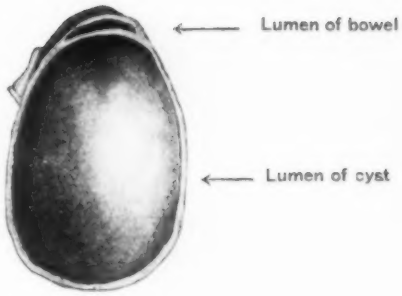

FIG. 3.—Cross section showing the compression of the bowel by the tense cyst.

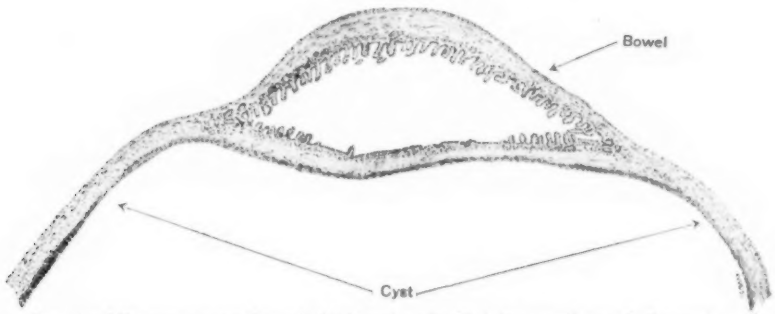

FIG. 4.—Microscopic section of the bowel and adjoining portions of the cyst wall. Note the blending of the walls of the two structures.

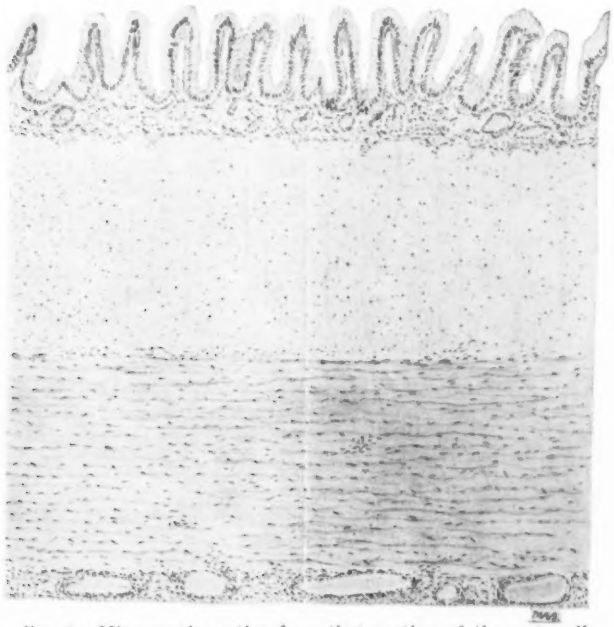

FIG. 5.—Microscopic section from that portion of the cyst wall farthest distant from the bowel. Note the almost exact similarity to the structure of the adjoining gut.

a doubt that such a cyst may occasionally persist to be recognized after birth as a "mesenteric cyst." The case which I have reported above seems certainly to belong to this group.

While the findings of Lewis and Thyng account for a certain number of mesenteric cysts, there is general agreement among those who have studied this question that most juxta-intestinal cysts, even when intramesenteric in position, have their origin in the vitelline or omphalo-mesenteric duct or in Meckel's diverticulum. By the end of the fourth week of fœtal life the connection between the mid-gut and the umbilical vesicle has narrowed down to a fine canal, the omphalo-mesenteric duct, which runs from the bowel to the region of the umbilicus. Normally this canal becomes obliterated at the beginning of the second fœtal month; when it persists as a blind duct emptying into the bowel it is known as Meckel's diverticulum. Meckel's diverticulum is said by Colmers to occur in about 2 per cent of all autopsies, but we do not know how frequently there may persist traces of the omphalo-mesenteric duct so small as to escape notice, and we are therefore unable to say just what proportion of individuals are born with a structure capable of developing by distension and growth into a juxta-intestinal cyst. The usual position of Meckel's diverticulum [13] is 43 inches above the ileo-cœcal valve, but it varies widely (11 to 120 inches); Lamb [14] found it on the duodenum 7 times, on the jejunum 14 times, and on the ileum 160 times, where in 91 cases it was less than 36 inches above the valve. Leichtenstern [15] believes that when the growth of the oral half of the mid-gut is delayed or fails most of the small bowel must develop from the aboral half, thus placing the omphalo-mesenteric duct and Meckel's diverticulum at a relatively high position on the small bowel, while an over-growth of the oral half of the mid-gut results in a relatively low position of the diverticulum; this explanation may suffice possibly to account for the wide variation in its position. The convex side of the bowel is usually described as the normal situation for Meckel's diverticulum, but it may simulate or possibly even occupy an intra-mesenteric situation. Roth [16] states that the diverticulum is sometimes not situated exactly on the convexity, but more to one side, and suggests that when this variation is marked and the attachment approaches the mesenteric border the diverticulum might become blended with the mesentery and simulate an intra-mesenteric position. Hennig [17] suggests that shrinkage of the mesentery of a laterally placed diverticulum might draw it down and into the mesentery. Colmers assumes that an intra-mesenteric position is possible, but offers no explanation, while Ruge is quoted by Bauer as regarding the concave side of the bowel as the normal situation for Meckel's diverticulum, though no reason is given for this view. Fitz [18] interpreted a case of double cœcum as the persistence of an omphalo-mesenteric duct which arose low in the bowel and occupied an intra-mesenteric position.

It is apparently established that Meckel's diverticulum or persistent remnants of the omphalo-mesenteric duct may give rise to a mesenteric or juxta-intestinal cyst along practically the entire course of the small bowel, though such an occurrence must be exceeding rare in the extreme upper limits of the bowel.

A mesenteric cyst of either of these two types (Lewis and Thyng's embryonic cysts and the omphalo-mesenteric duct) arises from the bowel whose structure it shares; such a cyst, when typical and unaltered by inflammation, presents certain features which are quite characteristic and easily recognized. The resemblance to the adjacent bowel may be little short of exact, in that there is found a mucosa with typical glands, goblet cells and ciliated cells, well-developed villi, a distinct submucosa, and two layers of smooth muscle placed at right angles to each other.

The picture, however, is usually not so clear. The widest variations from the typical are found in the epithelial lining of such cysts. Frequently the formation of villi is incomplete, their presence being merely suggested by more or less faintly marked rugæ or papillæ, or the lining may consist of a smooth epithelial surface which in no way suggests villi; it quite often happens that a single specimen exhibits all variations, as in the case described above, where intra-cystic pressure with stretching of the wall seemed to offer the simplest explanation. Secreting glands may be present in typical arrangement or may be represented merely by a suggestive grouping of the epithelial cells. Rarely there is nothing even suggesting a gland. The type of epithelial cell also varies widely. Quite commonly the predominant cell is high columnar, with goblet cells occurring more or less frequently; ciliated cells have also been found, but are not common. Kostlivy [19] found a jejunal mesenteric cyst lined by a single layer of ciliated cells, and cites from the literature the occurrence of a similar epithelium in three other enterogenous cysts, but one of which was mesenteric, however. It is interesting to note that Roth, who, in 1881, was the first to draw attention to enterogenous cysts, described one lined by ciliated epithelium.

There sometimes occurs an epithelium which is quite atypical, if the expression may be allowed; thus among 15 accurately described enterogenous cysts whose records I have studied there are two lined with stratified cuboidal, one with stratified ciliated, and nine with simple columnar epithelium, while three multilocular cysts, probably enterogenous in origin, are lined with a stratified epithelium. These variations from the type are readily explained by the embryological development of the gut. The stomach and small bowel both pass through an early stage when their epithelium is regularly stratified; during this period there may be in the stomach three layers of epithelial cells, while the duodenum of embryos of "30 to 60 days is normally more or less completely obliterated" [20] by epithelial proliferation. Kollman [21] states that the epithelium of the developing gastro-intestinal tract undergoes a regular progression from simple cuboidal to simple cylindrical to stratified cylindrical, from which finally develops the permanent layer of simple cylindrical epithelium, which is ciliated in the respiratory tract. The conditions governing the growth of a group of epithelial cells sequestrated during embryonic life would be quite anomalous; such a sequestrum might preserve the characteristics shown by its parent at the time of separation or might undergo retrograde changes or possibly might approximate a normal development. On these facts

most observers of an atypical epithelial lining have based an explanation of their findings.

The wall of a typical enterogenous cyst shows two well-developed layers of smooth muscle running at right angles to each other as they do in the bowel wall; such a cyst is of comparatively frequent occurrence. Not infrequently, however, one or both layers are poorly developed and irregularly arranged, and sometimes only a few isolated muscle fibers can be found scattered through the wall. At first glance the presence of smooth-muscle fibers in the wall of a mesenteric cyst would seem to indicate that such a cyst had arisen from the bowel; the question, however, is not so simple. In addition to the smooth muscle of the bowel wall there is certainly one and possibly two other sources from which smooth-muscle fibers in the wall of a mesenteric cyst may be derived. In the mesentery itself there regularly occurs a certain amount of unstriped muscle, portions of which might perhaps become incorporated in the wall of a cyst. Secondly, if we accept Klemm's idea that lymphatic mesenteric cysts are neoplasms, arising from misplaced portions of the mesoblastic layer, so-called mesodermoids, then we must expect smooth-muscle cells to occur regularly in the wall of such cysts along with the cells of the other tissues derived from the mesoblast. On the other hand, it is held by some that the absence of muscle cells in a cyst wall does not exclude an enterogenous origin, since these cells may have been destroyed by inflammation and replaced by scar tissue; Colmers, for instance, believes that even when "degeneration has destroyed the anatomical picture suggesting an intestinal origin the diagnosis of enterogenous cyst is justified if there is no proof to the contrary." Such an extreme position as that of Colmers can scarcely be accepted. No universally applicable law can be formulated to cover this question. We can certainly and positively affirm the enterogenous origin of any mesenteric cyst whose wall shows a well-developed musculature with an anatomical arrangement similar to that in the bowel. When, however, the picture is not so definite, or we find only a scant distribution of unstriped muscle lacking the typical arrangement, or perhaps no muscle at all, then we must seek farther for proof of an enterogenous origin. As a rule, the character of the lining will determine this point, but there will inevitably be instances in which we will be unable to finally decide the question.

The regional occurrence of enterogenous mesenteric cysts is apparently more or less distinctive. Since 1900 28 such cysts have been reported whose description is clear enough to permit classification. Of these 28 cases, including my case, all occurred in the mesentery of the small bowel, 7 occurred in the mesojejunum, 9 in the mesoileum, and 12 are said to have occurred in the mesentery of the small bowel, and hence probably in the mesoileum, since a higher location would have excited comment. The fact that all of these cysts were situated in the mesentery of the small bowel is striking; it may be merely a coincidence, but, nevertheless, it is suggestive when compared with the fact that the embryonic intestinal diverticula described by Lewis and Thyng all occurred on the small bowel. Stengel[22] found 15 of 18 "enteroid cysts" to occur in the mesoileum and regards this position as characteristic. In 22 cases the cyst was unilocular, in 4 multilocular, and in 2 cases there were multiple cysts; Deaver[23] describes the occurrence of a cluster of 4 cysts about the ileum, one being within and one resting upon the mesentery, the arrangement of the four being such as to produce partial obstruction. Von der Bogert[24] describes the occurrence of 13 cysts in the mesoileum, 12 of these being of rather small size. The relation of a mesenteric cyst to the bowel varies. The cyst may impinge upon the bowel directly from behind, so to speak, compressing it from its mesenteric toward its free border; usually, however, the cyst presents largely upon one side of the mesentery and may occupy a position quite at the side of the bowel. Not infrequently, as in my case, the cyst exercises enough pressure to cause a partial intestinal obstruction. Enterogenous cysts occur with much less frequency along the free edge of the bowel. In 2 of the above 28 cases the cyst was more or less pedunculated. During a hernia operation Broca[25] found the sac occupied by a non-adherent cyst which had its origin in the mesentery of the small bowel; the cyst was successfully enucleated and on examination its wall was found to contain smooth muscle. This case is apparently unique.

Concerning the size of such cysts little need be said. It is not infrequent to find a content of one or two liters or the cyst compared in size to an adult head, but as a rule enterogenous cysts do not seem to attain great size and are usually indefinitely described as "about the size of an egg."

Given two possible origins for enterogenous or enteroid or juxta-intestinal cysts, as they are variously styled, it would be of great interest to determine in each case whether the cyst represented the process of sequestration from the bowel, as described by Lewis and Thyng, or whether it developed from Meckel's diverticulum or the persistent remains of the omphalomesenteric duct. Manifestly this problem presents many difficulties and is often wholly impossible. There are certain general features, however, which may be pointed out. The intestinal diverticula and cysts described by Lewis and Thyng make their first appearance regularly in the upper bowel about the duodenum and jejunum, and, while the position of the vitelline duct and Meckel's diverticulum varies widely, yet its occurrence in the jejunum or duodenum is exceptional. It follows therefore that a situation along the extreme upper bowel argues for an origin by sequestration.

The process of sequestration occurs regularly at multiple points about the entire circumference of the bowel, while Meckel's diverticulum is a single structure. Multiple or multilocular cysts therefore suggest a sequestration origin, although it is obvious that by a process of subdivision a persistent vitelline duct might give rise to more than one cyst. Too much importance must not be attached to an intra-mesenteric position of the cyst, since it appears not at all unlikely that this position may be occupied frequently by Meckel's diverticulum. Bauer has pointed out that sequestration cysts are much older, genetically speaking, than those arising from the vitelline duct; this being the case, we would naturally find atypical variations in cell structure, notably of the epithelium, more generally present in cysts of the former type. A remarkable case reported by Ahrens[26] illustrates this point very well. There was

removed from a retroperitoneal position on the right side a four-liter cyst, which resembled grossly an hour-glass stomach, and 26 cm. of adjoining bowel. Histologically the cyst wall showed the typical structure of stomach and bowel from mucous membrane outward, except that the epithelium consisted variously of stratified and simple cubical, stratified ciliated, high columnar and flat cells. The fluid contained in the cyst was weakly acid and digested egg albumen on the addition of HCl. There were multiple ulcers in the pseudo-stomach. J. W. Hall [27] and Roegner [28] also report cases bearing on this point. All three cases are probably sequestration cysts.

Of the secondary pathological processes affecting enterogenous cysts it is interesting to note that both Bauer and Colmers call attention to the occurrence of tuberculous ulcers of the cyst wall as well as of adenoma, carcinoma and spindle cell sarcoma. Suppuration rarely occurs in true cysts, though it is not infrequent in diverticula, structures whose genesis may be similar to that of the cysts and which may be readily confused with cysts.

The fact that a cyst of the mesentery has never been positively diagnosed before operation or autopsy is conclusive evidence that distinctive signs and symptoms do not exist or have escaped recognition. In considering enterogenous mesenteric cysts we may conveniently recognize two clinical divisions; the first group, about 50 per cent of the cases, includes those patients with acute complications, which are fatal unless given prompt surgical treatment, and the second group those whose symptoms are never urgent. This division is suggested because a rather large number suffer acute intestinal obstruction; and with such symptoms many mesenteric cysts have been chance findings during operation or autopsy. In this group enterogenous cysts appear in numbers disproportionately great as compared to other cysts of the mesentery; thus in 17 cases of enterogenous cysts with full histories we find acute obstruction 8 times or in about 50 per cent of the cases and usually in early life. Among 24 enterogenous cysts (including the above 17) 13 gave symptoms leading to surgical interference during the first ten years of life. Of these 13 cases 3 occurred in the first year, 3 in the third to fifth years, and 7 in the fifth to tenth years. My case was operated upon on the fourth day of life. Nasse reports operation on the sixth day. These are the only cases I find showing intestinal obstruction immediately after birth. Complete intestinal obstruction is usually due to volvulus. The explanation of this fact is not far to seek. Enterogenous cysts are commonly situated close to the bowel and not infrequently partially occlude its lumen; above the point of obstruction there results a compensatory hypertrophy of the muscle and exaggerated peristalsis. The size of the cyst is generally not extreme, being frequently compared to a hen's or goose's egg, but it is filled with fluid and is relatively heavy; thus we may roughly compare the cyst and adjacent sector of bowel to a suspended weight which is peculiarly liable to torsion, the power necessary to cause such torsion being supplied in part by the exaggerated peristalsis above the cyst. Among 35 cases of enterogenous cysts of the bowel or mesentery which Colmers collected, 19 required surgical treatment; 8 which were not operated upon died with symptoms of intestinal obstruction or peritonitis, and of eleven operative cases 4 died, giving a grouped mortality of 12 cases or 35 per cent. He records the fact that in no case was a correct diagnosis made before operation. It thus appears that about one-half of all enterogenous mesenteric cysts cause acute intestinal obstruction, generally a volvulus, and that this calamity frequently occurs during the first decade. There is evident a striking similarity to intussusception in symptomatology, years of incidence, and obscurity of diagnosis, which urges caution in too "conservatively" withholding operation from a case of ileus in early life in the hope that a supposed intussusception will be reduced.

The remaining half of the cases, the latent cases, are not uniform in either symptoms or signs. There is frequently noted a sense of abdominal fulness, pressure or dragging; there is often a visible tumor or abdominal enlargement. Abdominal pain is quite common, but of no characteristic type. In only one instance was it associated with diarrhœa. Many cases in this group, perhaps most of them, give a history of mild recurrent attacks of partial intestinal obstruction.

The physical signs of a mesenteric cyst may be very definite, but are not pathognomonic. The typical case shows a freely movable, globular, fluctulant mass, varying in size up to that of the adult head, situated in any one of the four quadrants of the abdomen, and capable of being manipulated through a very wide range of motion. This last point is usually emphasized; it is sometimes possible to move such a tumor over the entire abdomen. These are simply the signs of any pedunculated cyst within the abdomen and are found in certain pathological conditions of the ovary, gall-bladder and kidney as well. In an instance, observed by the writer, a congenital occlusion of the ureter had converted the kidney into a large cyst, which answered rather accurately to the signs just enumerated. On percussion of a typical cyst one may find an area of flatness surrounded by a zone of tympany, a sign which is frequently referred to as important; judging from the records, however, this is by no means a constant feature. As a matter of fact, a perusal of the reported cases discloses so great a discrepancy between the signs actually observed and the so-called "typical" signs that one suspects imagination of having played a large part in the declaration of the latter. The fact that a correct diagnosis seems never to have been made needs no comment. A history and physical signs which conform in a general way to those which have been briefly presented above may suggest a mesenteric cyst very strongly, but an absolute diagnosis before operation or autopsy cannot be made.

The treatment of enterogenous mesenteric cysts may be covered in a few words. In the group of acute cases one is dealing always with intestinal obstruction or peritonitis and the therapy must be directed toward these conditions primarily; the operation is an emergency. In the second group, viz: the latent cases, the operation is an elective one and choice is to be made between drainage, enucleation and resection. Drainage is attended by a very low primary mortality, but is apt to result in a persistent sinus, which will ultimately require excision and, hence, is not altogether desirable. Without differentiating the type of cyst dealt with, Coley [29] has

estimated the mortality following drainage at 6 per cent; this probably includes some deaths due to complications and seems a very high rate. Enucleation was done 10 times without a death in 16 cases of my series. This is undoubtedly the procedure of choice when it is feasible, but conditions may render it impracticable. The cyst may be part and parcel of the intestinal wall, as in my case, where it would have been impossible to enucleate without opening the gut. Enucleation of a cyst or multiple cysts may do irreparable damage to the circulation of the bowel, enforcing resection. Dense adhesions may render enucleation impossible without tearing the bowel. The cyst may occupy an intra-intestinal position, as in several reported cases, near the ileo-cœcal valve and even be drawn into the cœcum, simulating an intussusception; in such circumstances the bowel must be opened to expose the cyst. These conditions among others may compel resection. In 5 cases of resection in my series there were 3 deaths, a mortality of 60 per cent. So high a mortality is due partly to the serious condition of most of these patients, of which my case is an excellent example, but the figures serve in a general way to emphasize the relative safety of enucleation of the cyst as compared to resection of the bowel. Undoubtedly the best treatment is enucleation when it can be done without seriously injuring or opening the bowel.

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SALVARSAN IN PERNICIOUS ANÆMIA.[1]

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The scattered reports on the use of salvarsan in pernicious anæmia which have appeared in the last three years are conflicting and unsatisfactory. In many instances the observation was confined to a single case and the salvarsan was only an accessory to the other standard therapeutic methods. As there has been repeated warning expressed in the literature against the use of salvarsan in this disease, it seems worth while to give a brief review of our own experience and some from the literature.

Bramwell's series of seven cases is an important contribu-

[1] Read at a meeting of the American Society for the Advancement of Clinical Investigation, Washington, D. C., May 5, 1913.

tion. With "cure" or favorable results in four cases, it is the most notable of the arguments for the use of salvarsan. Leede's five cases with unfavorable results are not nearly so instructive, as four of the cases were admittedly moribund when the drug was tried. The other case did seem to be injured by the two injections given.

Friedlander's case, refractory to Fowler's solution, was rapidly restored after two doses of salvarsan, followed by sodium cacodylate.

Through the courtesy of Professors Barker and Thayer, the writer has looked over the records of seven cases of pernicious anæmia, treated in part with salvarsan, at The Johns Hopkins Hospital. Of these, one received 0.3 gm. and died 48 hours later from pre-existing myocardial degeneration, without any reaction or any material change in the blood count. This patient was considered moribund on admission. Another case died 18 days after admission, receiving one dose of 0.2 gm. 11 days before death. There was no considerable reaction and no drop in the blood count of more than 200,000 cells. The remaining five showed improvement with initial drop in the blood count, never more than 200,000. As these cases also received sodium cacodylate or Fowler's solution, they are of value only as showing that the drug may be given to such patients without serious risk.

In our own experience at the Baltimore City Hospital we have had four cases, all of whom showed favorable reaction to the salvarsan in the regeneration of the blood and relief of the symptoms. One was a most remarkable apparent cure of a patient in his fifth relapse, who was quite unresponsive to Fowler's solution and only showed a very slight regeneration after a long period (four months) of sodium cacodylate injections. Under intravenous administration of salvarsan, in doses of 0.3 gm. every four weeks, he showed a steady rise in blood count. The red cells, in sixteen weeks, rose from 500,000 to 5,000,000, the hemoglobin from 23 per cent to 90 per cent, and the patient was well except for the mild degenerative changes in the spinal cord. He worked in the hospital for six months, as elevator man, and then left for outside employment and has been lost sight of. During the treatment the patient contracted tertian malaria and his blood count fell, but rose again after quinine and the next dose of salvarsan. This patient had no history of syphilis, and also had a negative Wassermann reaction in serum and spinal fluid before any salvarsan was given.

Another case of pernicious anæmia, in which the nervous symptoms preceded the change in the blood picture by some months, was very responsive to salvarsan, his blood going from 1,100,000 to 3,400,000 red cells in twenty days after the first dose of 0.3 gm. intravenously, eventually reaching 4,800,000 with 85 per cent hemoglobin. This patient gave no history of syphilis and Wassermann reactions in serum and spinal fluid were quite negative.

In this case the patient seemed unable to sustain the higher level of blood formation and relapsed rather quickly, only to rise to approximately normal count again after two more doses. The blood picture, however, was never free from the qualitative changes of pernicious anæmia. After several rallies of this kind, covering a twelve-month, in which the subjective state was but little altered, the patient died of an intercurrent bronchopneumonia. Autopsy: typical changes of pernicious anæmia, with funicular degeneration in the cord.

The other two cases, both negative for syphilis, received but one injection each, and were greatly improved, with an average rise of 2,000,000 red cells when they left the hospital.

In our own cases and those from The Johns Hopkins Hospital there was, as a rule, a rather sharp febrile reaction, lasting six to twelve hours after each injection, in contrast to the very mild, or absent, reaction in syphilitics. All precautions were taken with regard to technic, *wasserfehler* and size of dose.

The results to date would certainly justify the further use of salvarsan in pernicious anæmia with special attention paid: (a) to the question of syphilis in the patient; (b) to the influence of salvarsan when given alone; (c) to the effect on arsenic refractory cases; and (d) permanency of the results.

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ETIOLOGY OF ARTERIOSCLEROSIS.*

By CHANNING FROTHINGHAM, JR., M. D., Boston, Mass.

Many different conceptions as to the nature of arteriosclerosis exist in the medical literature. To review them would be interesting, but outside the scope of this paper. In the chapter on diseases of the blood vessels, in his book which is about to be published, Dr. Mallory says: "Arteriosclerosis is the term applied to the more or less chronic lesions of the aorta and arteries or to the end products of these lesions. It is the end result of various toxic, nutritional, and infectious lesions involving the arterial system. It corresponds to sclerosis of the liver, kidney, and other organs. It is usually of toxic origin, but may be the result of infection (syphilis). The two forms may be and often are combined. Disturbances of nutrition may play an important part, more particularly in the smaller vessels (kidney for example)." This description in my opinion covers most satisfactorily the various pathological conditions of a chronic character found in the blood vessels. It separates from arteriosclerosis the numerous acute pathological processes in the arteries and any physiological changes in thickness of the vessels at different ages which have often been reported.

Mallory, Klotz, and others emphasize the point that it is impossible to say from the end product of these arterial lesions

* Read at a meeting of The Johns Hopkins Hospital Medical Society, May 5, 1913.

just what was the cause of the initial injury, except in certain rare types. Arteriosclerosis in any individual may be the result of an extensive injury to the arterial system produced in a short time, or simply the accumulation of a number of small injuries to the arteries scattered over a period of years. Therefore, in order to find the factors which produce arteriosclerosis, one must look for the etiological factors of any acute arterial lesions which will leave a scar on entirely or partially healing.

An important and as yet undecided question is whether the arterial wall adjacent to a healed or partially healed area suffers from disturbance of nutrition and therefore degenerates. If such is the case, a vicious sequence will be started after any permanent injury to the vessel wall, which will make the condition a progressive one. Before discussing the different agents that produce acute arterial lesions, I will present Dr. Mallory's classification of the different types of arterial degeneration and necrosis. His description is based upon the initial step in the process in each case, and it is evident that arteries respond in the same manner to a variety of toxic agents. The same active agent, however, may cause different types of lesions in the vessels of different organs. Hyaline formation is common in the spleen under a variety of acute and chronic conditions, but nothing similar occurs in the aorta. Also lesions that start the same may, as they progress, become quite different in appearance. In the larger arteries one layer may be involved without the others, but in the smaller all layers are usually involved together.

Dr. Mallory says, "Injury to the arterial wall manifests itself by two forms of retrograde change, by an accumulation of fat in the injured cells or by necrosis. These may be complicated by fibrine formation, hyaline transformation, or by the deposit of lime salts."

Any of the cells composing the vessel wall, fibroblasts, smooth muscle cells, or endothelial cells may be involved. Cells with fat in them may under improved conditions use up the fat and return to normal. A necrotic cell may call forth no reaction, if it dies suddenly and contains no fat. It will gradually be absorbed. Therefore these two types of acute lesion may heal without leaving a permanent scar.

Free fat from cells that have died or necrosis of any extent will call forth a cellular reaction of endothelial leucocytes, and if extensive enough polymorphonuclear neutrophiles, and usually a proliferation of fibroblasts so that on healing a permanent scar remains. Occasionally there does not seem to be enough injury to call forth a marked reaction, and areas with considerable fatty degeneration persist with little attempt at repair. In other places the tissue probably is not able to completely recover, but remains in a state of degeneration with moderate reaction. In the distributing arteries necrosis may occur in the media entirely independent of the intima. In these cases fat and lime salts may be present without any reaction about them. Chronic and acute arterial lesions like all other lesions may be very irregularly and unaccountably distributed throughout the body.

I will now take up a list of the agents between which and the arterial lesion a direct relation seems to be fairly well established, either by a study of autopsy material, animal experimentation, or clinical observation. Before the discovery of the treponema pallida it was a well recognized clinical observation that patients presenting a history of syphilis frequently showed evidence of arteriosclerosis. Fremont Smith reported a case of a boy of 12 years with severe general sclerosis which he felt was surely due to congenital syphilis. Recently the discovery of the spirochetæ of syphilis by Wright and Richardson and others as causative agents in lesions that leave a permanent scar in arteries proves conclusively that this organism plays a part in the cause of arteriosclerosis.

The tubercle bacillus may produce arterial lesions by extension from neighboring lesions, or by infection of the wall from the inside. Wooley recently reported such a case and referred to others. Of course such lesions would be rare and play little part, even if they healed, in causing a general sclerosis. In order to see if the tubercle bacillus had a soluble toxin which might produce a more general arterial lesion, I studied six fatal cases of tuberculosis under 25 years of age. Tissue from the spleen, aorta, and kidneys was examined. One of the cases, an infant under two years of age, showed no arterial lesions. The others in one or more organs showed lesions which would leave a permanent scar on healing. Of course it is impossible to say that these were due to the tubercle poison more than to metabolic poisons which might have arisen from the general weakened condition. This is especially difficult to decide since the character of the lesion would presumably be the same in either case.

The leprosy and glanders bacilli have been found in acute arterial lesions, but those diseases are so uncommon that they can play very little part in the cause of arteriosclerosis, and so I will only briefly mention them.

Flexner reported acute arteritis in typhoid fever. In two cases under 25 years of age I found slight degenerative lesions in the kidney and splenic arteries from which recovery was possible, and slight lesions of a permanent character in the aortæ. Here also the question justly may be raised as to whether there is any specific action from the typhoid toxin which causes these lesions. Experimentally Klotz claims some positive results in the aorta from injection of typhoid bacilli into rabbits. I could not produce any lesions from the injection of typhoid vaccines in guinea pigs. Clinically Thayer and Brush have found that a series of people who have had typhoid fever present more palpable radial arteries than a series of people who have not, who live in presumably the same circumstances. The direct evidence against the typhoid bacillus as a factor in the cause of arteriosclerosis is somewhat questionable.

Near the site of the diphtheria membrane I have found extensive necrosis of the vessel walls which will undoubtedly leave a permanent scar on healing. In looking for a generally distributed lesion in 10 cases under 25 years of age none were found in 3 cases under 2 years of age. In the other 7 cases the splenic vessels consistently showed a lesion which would leave a scar on healing, and the kidney and aorta occasionally did. Wiesel, in a study of the vessels in children dead of diphtheria, reports very general arterial lesions.

Experimentally Thérèse, Pernice, Boinet and Romary, and Klotz found lesions in the rabbit's aorta after injection of diptheria toxin similar to arteriosclerosis in man. At the present time it is only fair to state that the relation between this type of disease in rabbits' aortæ and the substances injected is not well established. My feeling is that for the present this type of arterial lesion in rabbits had better be considered as a spontaneous lesion and not the result of the substances injected. Recently I have produced an extensive lesion in some of the smaller vessels of the rabbit's kidney with diphtheria toxin which would undoubtedly leave a permanent scar on healing. Clinical evidence in regard to the relation of diphtheria toxin and arteriosclerosis is lacking.

In cases infected by any one of the group of bacteria including the pneumococcus, staphylococcus, streptococcus, and the diplococcus of acute articular rheumatism Wiesner, Wiesel, and Symnitzky have all reported evidence of arterial degeneration in those of young age. Mallory and others have found marked arterial necrosis with fibrin formation and cellular reaction scattered throughout the different organs in such cases. Out of 48 cases dying from acute infection I found in one or more organs such lesions in some of the arteries in 8 cases. In addition to these cases with the extensive localized lesion I studied 18 cases under 25 years of age infected with these organisms to see if there was any diffuse lesion attributable to their toxins. I found practically no lesions in children under 2 years of age. In the others there was some evidence of lesions in the arteries of the spleen and aorta which would produce a permanent change on healing, but very little in the kidneys. Saltykow, Crocq, Klotz, and others have obtained positive results in rabbits and guinea pigs with such toxins, but it seems to me necessary to have the work confirmed. By the injection of staphylococcus vaccine in young guinea pigs, I was able to keep them from being as large as the controls, but did not produce any arterial lesions. Clinically Thayer and Brush have found that the radial arteries of people, who have had these infections, especially rheumatism, are more readily palpable than those of controls. It is certain therefore that these infections may cause localized arterial lesions, but the evidence in regard to their causing diffuse arterial disease is lacking.

In poliomyelitis Robertson and Chesley have found local arterial degenerations in the spinal cord, and in influenza meningitis Rhea has reported a lesion in the meningeal vessels. There have been practically no other observations with these infective agents.

From these different findings it is evident that certain of the infections with organisms produce quite marked injury to arteries either by their presence or by the action of their concentrated toxins. These lesions are usually limited in their distribution. In addition many of the acute infections have associated with them more or less diffuse arterial lesions of a chronic type in one or more organs. As investigation continues along these lines it seems probable that more organisms producing infectious diseases will be found to produce arterial lesions.

Fremont Smith quotes many authors who have found extensive sclerosis in young people and fœtuses without apparent cause. I studied the clinical histories of two cases of marked sclerosis, aged 22 and 16 years, in order to see if any light could be thrown on the cause from this source, but there was no evidence of any one particular cause that might have produced the condition.

The relation between non-infectious toxins and arteriosclerosis is clinically and pathologically more marked, but yet more difficult to prove, because it occurs chiefly in older people in whom other factors might well take a part. Foremost among the non-infectious toxins are either an excess of the normal products of metabolism, or abnormal elements produced by a disordered metabolism. One or both of these appear during the course of a chronic nephritis. That the poisons during a chronic nephritis produce lesions in the vessels of a permanent nature seems certain from the extent of involvement seen post mortem in patients with chronic nephritis, as compared with others from the same environment dead from other causes. I attempted, by having both kidneys removed from rabbits by Dr. Loder, to produce arterial lesions by uræmic poisoning. The animals lived long enough, about 2 days, for fatty changes to appear in the cells of several organs, but the blood vessels appeared normal. Clinically chronic nephritis cases show evidence of arteriosclerosis.

In chronic jaundice cases extensive fatty changes have been reported in the aorta which were so marked that a relationship between it and the bile seems justifiable. Dr. Minot and I endeavored to produce vascular changes in rabbits by the injection of bovine bile both intravenously and intraperitoneally. Although in some of the animals fatty changes were present in some of the parenchymatous cells no arterial lesions were obtained. Whether the fatty changes produced by bile go on to permanent arterial changes has not been definitely settled.

Clinically and at autopsy, cases of lead poisoning usually show extensive sclerosis of the arteries. They also usually show nephritis to some extent. It is therefore difficult to place the blame for a specific action upon the lead. I fed young rabbits lead acetate over a period of 2 to 3 weeks in such amounts that some died and others were killed while quite sick. These animals showed no arterial lesions.

In addition to the acute infectious toxins and organisms which may produce arteriosclerosis, must be mentioned, therefore, the poisonous products of metabolism remaining in the system due to faulty elimination. Lead also must be borne in mind as a probable etiological factor.

Whether cells wear out under perfectly normal conditions from old age is a question in regard to which there is no definite clinical, post mortem, or experimental proof. It must not be forgotten, however, as an etiological factor in the production of sclerosis of the arteries.

In would probably simplify the situation to stop here and omit from consideration any other possibilities as a cause for arteriosclerosis. It is impossible, however, not to mention some of the numerous agents accused of causing arteriosclerosis in the literature. These can be best grouped as toxic and non-toxic agents.

Of the toxic ones those receiving the most attention at the present are the poisons absorbed from the alimentary tract as a result of proteid decomposition. Metchnikoff has worked out a theory that some of the scleroses in organs and vessels met with in old age are the result of the absorption of indol, paracresol, etc. He reports some positive experimental results with the injection of paracresol, and Dratchinski reports arterial scleroses after injection of indol in animals. To me the work is not in the least convincing, and the application of these theories to man seems to be entirely theoretical, without a firm experimental, clinical, or pathological basis to stand upon.

Clinically the use of alcohol has been shown pretty suggestively by Thayer and Brush to be a factor in the cause of arteriosclerosis. Pathologically Cabot has offered some very convincing statistics to show that alcohol does not play any part in causing arteriosclerosis. Experimentally little has been done with alcohol in arterial work.

There has been evidence brought clinically against nicotine as an agent in producing arteriosclerosis. Hochwart of Vienna has written on the subject. Experimentally Adler and Hensel have had some positive results in rabbits. The direct proof that nicotine may cause arterial lesions in man is lacking.

Most of the writers on this subject speak of the relation between gout and arteriosclerosis although there is little pathological or experimental evidence to back it up.

The non-toxic factors mentioned as producing arteriosclerosis are heredity, changes in blood pressure, and overwork. Since arteriosclerosis is not a specific disease, but a summation of injuries from one or more sources, it is not clear to me how heredity can be looked upon as a cause of sclerosis except in that one person's vessels are less capable of withstanding infections and metabolic poisons than others. Such proof is exceedingly difficult to produce, as, to do so, it would be necessary to be able to measure the degree of virulence of the individual toxin. However, Osler, Brill and Libman, and others speak of the part heredity may play in this disease.

Changes of blood pressure have been held accountable for arteriosclerotic lesions. Romberg even mentions that emotions may cause arteriosclerosis by frequent oscillations of pressure. To study this point substances that either raise or lower blood pressure have been injected into animals. Foremost among them are adrenalin and nicotine. Clinically and from post mortem examinations there has been practically nothing in the way of direct evidence against the substances capable of changing blood pressure. Experimentally since the first report of Josue in 1903 adrenalin and other blood raising or lowering drugs have been administered chiefly to rabbits, and occasionally to other animals. Adler and Hensel have worked with nicotine. A great many positive results have been obtained. The question always comes up as to the relation between these results and the spontaneous disease in rabbits' aortæ. At the present it seems fair to state that there is no proof that such substances produce arterial changes of a permanent character in man.

In regard to overwork there has been practically no experimental study reported. Thayer and Fabyan have shown the pathological changes in the radial arteries from age and strain. It has been observed clinically that motormen who are on their feet a long time may show markedly sclerosed femoral arteries and blacksmiths may show more sclerosis in their right arms than in their left. Thayer and Brush have found the radials more palpable in those who indulge in heavy physical labor. Klotz considers progressive medial disease in the peripheral vessels the result of muscle fatigue and nutritional disturbance.

As one goes down the list of substances accused of producing arteriosclerosis the proof in support of the claims becomes less and less. It seems to me, therefore, that, until some more definite evidence is offered against any agent it is best to consider that the only causative factors in the production of arteriosclerosis are the products of normal or abnormal metabolism retained in the body through faulty elimination, and the acute infections. Justifiable suspicion, however, points towards a number of the factors mentioned above.

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